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THE BLOOD PICTURE OF THE SOUTHERN ARMYWORM (PRODENIA ERIDANIA) [1]

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INTRODUCTION

The blood cells, or hemocytes, of insects form part of a defense mechanism against bacteria and other foreign bodies. They are analogous to the leucocytes of the vertebrates. Recent work on the cockroach (*Periplaneta americana* (L.)) has indicated that interference with the functions of the hemocytes may affect the resistance of the organism to certain insecticides in the usual laboratory tests (*33*)[2]. Oral administration of some insecticides is followed by abnormal changes in the hemocytes of the southern armyworm (*Prodenia eridania* (Cram.)) (*35*). The functions of different kinds of insect blood cells are but little understood, however, and extensive information regarding their physiological roles cannot be obtained until the various types of these cells in representative species are known.

Of the more comprehensive classifications of insect blood cells, those reported by Hollande (*7*) and Paillot (*20*) have been the most generally accepted (*29, p. 234*). Hollande (*8*) finally rejected his own classification and tentatively accepted that of Paillot.[3] Paillot's classification, however, is too simplified and of too general a nature to describe adequately the hemocytes of *Prodenia eridania* and, perhaps, of many other species as well. A single classification sufficiently comprehensive and at the same time sufficiently detailed to describe all the types of hemocytes that occur in different species, particularly those of different genera, does not yet exist. If such a classification is to be achieved at all, it must be preceded by and based upon more intensive studies of the types of blood cells in a number of species representative of different orders.

In this investigation the blood cells of the lepidopteran *Prodenia eridania* were identified and classified, differential blood-cell counts were made, variations in the blood-cell picture at different stages of development of the insect were determined, and certain relationships between the different types of blood cells were outlined.

METHODS

INSECTS EXAMINED

The insects used in this study were reared from eggs in cages kept at about 24°–30° C. throughout the year, except for occasional higher temperatures in summer. The larvae were fed on freshly grown green leaves of turnips, collards, or both. Usually the young larvae were reared on turnip leaves and the older on collard greens. As a rule

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they ceased eating shortly before entering the ground. The imagoes were given a sugar solution.

The instars were identified by means of head-capsule measurements. By means of such measurements Mayer and Babers (*15*) have shown that the southern armyworm larva develops through six instars. In the present work larvae were selected whose head-capsule measurements lay within one standard deviation of the mean for a desired instar. The head-capsule widths of the various instars that were selected, therefore, lay within the following ranges: (I) 0.274 to 0.306, (II) 0.433 to 0.507, (III) 0.704 to 0.836, (IV) 1.027 to 1.193, (V) 1.512 to 1.808, and (VI) 2.239 to 2.581 mm.

Age within a given stage of development was determined for many of the insects, including those whose blood smears were differentially counted. The ages of the first instars, pupae, and imagoes were reckoned from the times of hatching, pupation, and emergence, respectively; those of the other instars were reckoned from the time of the molt. Successive ages of the prepupae were designated as (1) when the larva was entering the ground, (2) when the insect was beginning to taper, and (3) when it was well tapered. In this paper the period of metamorphosis is considered to include the prepupal and pupal stages.

The sex of the larvae could not be identified. The sex of the pupae was determined from markings analogous to those described by Jackson (*12*), and that of the imagoes by examination of the external genitalia.

BLOOD SAMPLING AND SMEARING

The sampling and smearing of the hemolymph of small and large larvae, pupae, and imagoes have already been described (*34*). Unless otherwise specified, the smears were made from insects that had been immersed for 5 to 10 minutes in water at 60° C. to prevent the hemocytes from changing form, for it was desired that they retain as closely as possible the form they had in the circulating blood.

STAINING

The air-dried smears were stained with Wright's blood stain,[4] which was applied by the Wright nicotine-oxalic acid procedure (*30*). Some of the smears, however, were fixed with methyl alcohol, flooded with equal parts of the Wright's stain and a 2.5-percent aqueous solution of nicotine that had not been redistilled, dipped in 70-percent ethyl alcohol to remove excess stain, and flooded briefly with oxalic acid solution to give a more brilliant differentiation. In other cases this procedure was followed except that oxalic acid was not used and differentiation was obtained by successive quick dips in 70-percent alcohol. The staining time was adjusted to meet the different requirements of the larval, pupal, and imaginal smears.

Although the results reported in this paper are based upon smears colored with Wright's blood stain, other stains were used incidentally. Giemsa's stain gave essentially the same results as Wright's.

In making color photographs (Kodachrome), a $93\times$ objective (oil immersion) and a $10\times$ ocular were used except in photographing the cells in the right-hand part of plate III, *A*, when a $45\times$ objective (dry) was used. Thus, all except the cells last specified are shown magnified to the same extent.

DEVELOPMENT OF THIS CLASSIFICATION

An attempt was made to develop this classification on as nearly quantitative a basis as practicable. Smears from a large number of insects in different stages of development were studied. What seemed to be different kinds of hemocytes were noted and arranged in an initial classification, which was used as a basis for making differential counts. An attempt was made always to classify all the cells found during a count. Cells were observed that could not be identified by the use of this classification, and eventually the latter was reconstructed to form a second one, which was used as a basis for further differential counting. Eleven successive classifications were thus made, used, and eventually reconstructed. The classification herein reported represents the twelfth; all the earlier classifications were discarded.

The differential counts were made in the way usually employed with blood smears. Hemocytes in a smear were found randomly and identified. An attempt was made to include at least 400 cells in each count, but this number was not always attained, especially from young larvae, old pupae, and imagoes whose smears contained very few cells. Since blood cells tended to be exceedingly scarce in the smears from old pupae, counts were not made from the oldest pupae.

Fusiform-nonfusiform (F/N) ratios were determined as described on page 23.

BASIS FOR NAMING CLASSES AND TYPES OF CELLS

Names for the classes and types of hemocytes were chosen as nearly as possible on the following basis. When a class or type of hemocyte appeared to be identical with one reported in a previous classification, it was given the name used previously, as in the case of the proleucocytes and oenocytoids. Names for most other kinds of hemocytes were selected to indicate their morphological appearance rather than a presumed genetic relationship. Microcytes, podocytes, nematocytes, and spheroidocytes are examples of such names. Certain cells that might or might not be real transitional forms, but resembled certain distinct types, were given the name of the type that they most resembled with the suffix "oid"—for example, nematocytoids and cystocytoids. In pseudoenocytoid the prefix "pseudo" was used because the name already ended with "oid." For certain types within a given class which bore a very apparent developmental relationship to each other, the names indicate a serial but not necessarily a genetic relationship—for example, microplasmatocyte, mesoplasmatocyte, and macroplasmatocyte. The prefix "eo" in eoplasmatocyte shows that the cells occur mostly in the early instars. Within the classes of podocytes and cystocytes, however, certain type names do indicate a developmental relationship; for example, propolypodocyte implies that the cell is a developmental precursor of the polypodocyte.

RESULTS

CLASSIFICATION OF BLOOD CELLS

The classification of the blood cells of *Prodenia eridania* is given in table 1. The variants of each type are shown, and the types are combined into classes. The symbols given for the classes and types in this table are also used on the plates.

TABLE 1.—*Classification of the blood cells of Prodenia eridania*

Class			Type			Variant	
No.	Name	Symbol	No.	Name	Symbol	No.	Kind
I	Proleucocytoids	a	1	Microcyte	aa	0.1	Fusiform.
						0.2	Nonfusiform.
						0.3	Fusiform, deeply basophilic.
						0.4	Fusiform, lightly basophilic.
						0.5	Fusiform, amphophilic.
			2	Proleucocyte	ab	0.6	Nonfusiform, deeply basophilic.
						0.7	Nonfusiform, lightly basophilic.
						0.8	Nonfusiform amphophilic.
II	Smooth-contour chromophilic cells (chromophiles)	b	3	Liocyte	ba	0.9	Deeply basophilic.
						0.10	Lightly basophilic.
			4	Liocytoid	bb	0.11	Deeply basophilic.
						0.12	Lightly basophilic.
III	Oenocytelike cells	c	5	Pseudoenocytoid	ca	0.13	
			6	Oenocytoid	cb	0.14	
IV	Plasmatocytes	d	7	Macroproleuoccytoid	da	0.15	
			8	Eoplasmatocyte	db	0.16	
			9	Eoplasmatocytoid	dc	0.17	
			10	Microplasmatocyte	dd	0.18	Fusiform, deeply basophilic.
						0.19	Fusiform, lightly basophilic.
						0.20	Nonfusiform, deeply basophilic.
						0.21	Nonfusiform, lightly basophilic.
			11	Mesoplasmatocyte	de	0.22	Fusiform, deeply basophilic.
						0.23	Fusiform, lightly basophilic.
						0.24	Nonfusiform, deeply basophilic.
						0.25	Nonfusiform, lightly basophilic.
			12	Macroplasmatocyte	df	0.26	Fusiform, deeply basophilic.
						0.27	Fusiform, lightly basophilic.
						0.28	Nonfusiform, deeply basophilic.
						0.29	Nonfusiform, lightly basophilic.
			13	Elongated plasmatocyte.	dg	0.30	Very fusiform.
			14	Multiramous plasmatocyte.	dh	0.31	
V	Podocytes	e	15	Propolypodocyte	ea	0.32	
			16	Polypodocyte	eb	0.33	
VI	Vermiform cells	f	17	Nematocytoid	fa	0.34	Extremely fusiform.
			18	Nematocyte	fb	0.35	Wormlike.
			19	Multiramous vermiform cell.	fc	0.36	
VII	Cystocytes	g	20	Cystocytoid	ga	0.37	Fusiform.
						0.38	Nonfusiform.
			21	Neocystocyte	gb	0.39	Fusiform.
						0.40	Nonfusiform.
			22	Paleocystocyte	gc	0.41	Fusiform.
						0.42	Nonfusiform.
			23	Cystocytic plasmatocyte.	gd	0.43	Fusiform.
						0.44	Nonfusiform.
VIII	Spheroidocytes	h	24	Spheroidocytoid	ha	0.45	
			25	Orthospheroidocyte	hb	0.46	
			26	Metaspheroidocyte	hc	0.47	
			27	Paraspheroidocyte	hd	0.48	
IX	Eruptive cells	i	28	Rhegmatocytoid	ia	0.49	Intact.
						0.50	Erupting or erupted.
			29	Rhegmatocyte	ib	0.51	Leucovesicular.
						0.52	Eosinovesicular.
						0.53	Erupting.
						0.54	Erupted.
X	Degenerating	j	30	Achromophile	ja	0.55	
			31	Hyaline cell	jb	0.56	
			32	Degenerating cell	jc	0.57	

The various cell shapes are illustrated in figure 1. The shapes
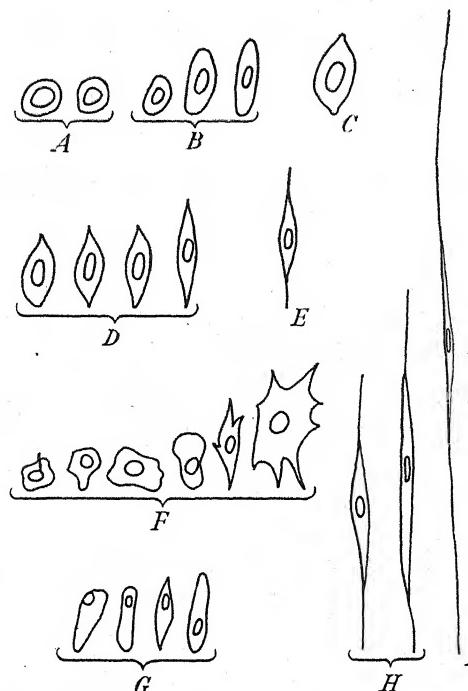
FIGURE 1.—Outline drawings to illustrate meanings of terms referring to cell shapes.

graduate one into the other and have to be separated by arbitrary boundaries. They were distinguished as follows in the present work:

Round, completely circular or nearly so (*A*).
Ovoid, very slightly to extremely (*B*).
Fusiformoid, tending to have but not actually having pointed spindle ends (*C*).
Fusiform, having two spindle ends or one true spindle end and one end not quite of spindle shape (*D*).
Very fusiform, elongated cells varying between *D* and *H* (*E*).
Polymorphic, having various, irregular shapes (*F*).
Elongated oenocytoids, having various elongated shapes (*G*).
Extremely fusiform, having spindle ends still longer than very fusiform cells and bodies in various degrees of elongation (*H*).
Vermiform, having the most elongated spindle ends and bodies and similar cells that taper more gradually from nuclear region (*I*).

Unlike the nuclei of vertebrate leucocytes, the nuclei of the southern armyworm blood cells are generally eosinophilic. When properly stained most of them exhibit a bright-red color, which may be more or less obscured by basophilic cytoplasm or cytoplasmic inclusions. In the descriptions that follow it is assumed that the nuclei are eosinophilic unless otherwise specified. The nuclei are usually punctate. Apparent nuclear-cytoplasmic transfer (*31*) is evidenced occasionally.

The cytoplasm is generally basophilic (blue), although it may be amphophilic (gray) or slightly eosinophilic (red). The intensity of the basophilia varies from cell to cell but tends to be uniform in a single cell, the depth of color apparently differing with the thickness of

the cytoplasm. The cytoplasm is seldom absolutely homogeneous, but ranges from approximately homogeneous to a condition of marked but fine vacuolization. Cytoplasm may also contain gross, colorless vacuoles, the occasional colorless or nearly colorless cysts of the cystocytoids and neocystocytes, the colorless spheroids of the spheroidocytes, and the colorless vesicles of the eruptive cells. Usually only one or two gross vacuoles are found in a single cell.

None of the southern armyworm hemocytes exhibit distinct cellular membranes when seen in dried smears stained with Wright's blood stain as herein reported. References to distinctness of cell boundaries do not imply that cell membranes are visible, but only indicate whether or not the cytoplasmic edge of the cell stands out in contrast to the plasma background.

Some of the hemocytes undergo passive-active transformation, indicated by the rounding up of fusiform cells, the spreading of cells in the smear, and the appearance of ectoplasmic differentiation or irregularities. Frequently these changes are accompanied by a tendency of the cells to agglutinate.

In general, the hemocytes of the earlier instars are smaller than those of the later instars. The cell sizes given in the following descriptions of types are based upon actual measurements but are intended to be suggestive only. A complete study of the sizes of the different types of cells would involve the measurement of more cells than has been practicable in this study. Cell sizes are expressed in microns (μ); "s.d." indicates the range for the smallest diameter and "l.d." that for the longest diameter.

CLASS I. PROLEUCOCYTOIDS

(Proleucocytelike cell)

The proleucocytoids are small cells ranging in shape from round to polymorphic or fusiform. They are separated according to size into the minute microcytes (pl. I, A, aa_{1-2}) and the larger proleucocytes (B, ab_{3-4}). They are nearly always basophilic and usually stain a moderately deep blue. Occasionally (B, ab_{3-4}) they are intensely basophilic and resemble small liocytes or liocytoids. Infrequently they are amphophilic or may show a slight, dull eosinophilia.

TYPE 1. MICROCYTE

(Very small cell)

The microcytes are minute cells. Their tiny bit of cytoplasm shows little or none of the finer colorless vacuolization characteristic of the plasmatocytes and generally contains no gross vacuoles. These cells may be round, fusiform (pl. I, A, aa_1), or polymorphic, and have an average diameter of not more than 4.5μ (arbitrary limit) when not fusiform.

In Plate I, A, the nuclei of the cells at aa_1 and aa_2 are obscured by cytoplasm. The polymorphic cell at aa_2 could be a recently divided daughter cell, formed by mitotic division of, say, a proleucocyte.

TYPE 2. PROLEUCOCYTE

The term "proleucocyte," assigned by Hollande (?) to small cells having relatively large nuclei and small amounts of cytoplasm, has been used with the same meaning by other authors and is so employed here, except that such cells having an average diameter of 4.5μ or less are considered to be microcytes. The nucleus is usually round or nearly round, but it may vary to markedly ovoid. The cytoplasm may stain from very deep to light blue. It contains few to no gross colorless vacuoles and little or no finer vacuolization. The nuclei of most of the proleucocytes are visible, but they may be variably obscured by overlying cytoplasm, especially in some of the very deeply basophilic cells. Most of the proleucocytes are round to polymorphic, but occasionally cells that could be classed only with

the proleucocytes were fusiform. Nonfusiform cells, s.d. 4.5μ–8.6μ, l.d. 4.5μ–9.2μ; fusiform cells, s.d. 2.1μ–5.4μ, l.d. 6.4μ–12.9μ.

Plate I, *B*, shows proleucocytes from a first (ab_{1-2}) and a fifth (ab_{3-4}) instar. Cell ab_1 would probably have developed into a microplasmatocyte, cell ab_2 into a liocyte. Cells ab_3 and ab_4 are larger and more like liocytes, and possibly represent development into liocytes, or into microplasmatocytes as at *dd*.

No distinct size or form interval separates the proleucocytes from the microcytes, liocytes, liocytoids, microplasmatocytes, spheroidocytes, rhegmatocytoids, or pseudoenocytoids. Occasionally cells seemed to be transitional between the proleucocytes and the mesoplasmatocytes or the nematocytes.

CLASS II. SMOOTH-CONTOUR CHROMOPHILIC CELLS (CHROMOPHILES)

The smooth-contour chromophilic cells have either a very smooth periphery or a cytoplasm that stains very deeply with methylene blue, or both. The typical liocyte has a somewhat turgid aspect, an unusually smooth periphery, and a very deeply basophilic cytoplasm, which surrounds, overlies, and tends to obscure the nucleus. The typical liocytoid is also deeply basophilic, but its periphery is not completely smooth. The intensity of its basophilia ranges from that of the deepest liocytes to that of the more deeply stained plasmatocytes. Although most of the smooth-contour chromophiles are deeply basophilic, a few lightly basophilic cells are also included in this class.

The liocytes and liocytoids, like the plasmatocytes, tend to be more irregular in shape and more distinctly vacuolized in the adult than in the larva.

TYPE 3. LIOCYTE

(Smooth cell)

The nuclei of the liocytes range from round to ovoid in shape, may be variably obscured by cytoplasm, i. e., to different degrees within a cell as well as in different cells, and when overlaid by very deeply stained cytoplasm may appear almost black. The cytoplasm has little or none of the finer colorless vacuolization characteristic of the plasmatocytes and contains few to no (usually no) gross colorless vacuoles. The cell contour is distinct and may have an egglike smoothness (pl. I, *C*, ba_{2-4}). The cell may be round, ovoid, smoothly irregular, or, infrequently, fusiformoid. The liocytes are thicker than most of the proleucocytes and plasmatocytes. Some of the smallest liocytes are practically indistinguishable from some of the very deeply stained proleucocytes (*B*, ab_{3-4}) and may represent transitional stages. Occasionally liocytes resemble pseudoenocytoids. Very deeply stained liocytes may appear as smooth, opaque black bodies. S.d. 4.5μ–8.6μ, l.d. 5.3μ–11.8μ.

Typical liocytes are shown in plate I, *C*, ba_{1-4}, and *D*, ba_{1-2}.

TYPE 4. LIOCYTOID

(Liocytelike cell)

The liocytoids contain round to ovoid nuclei, which may be variably obscured according to the thickness of overlying cytoplasm and the intensity of the stain. Examples of this type are shown in plate I, *C*, *bb*, and *D*, bb_{1-4}. Some of the chromophilic cells of the large groups shown at the left in *G* are also liocytoids. The cytoplasm may stain from light to deep blue and is usually a more intense blue than that of the deeply stained plasmatocyte. A portion or none of the cell periphery may exhibit a liocytelike smoothness. The regions of the cytoplasm associated with the part of the cell surface that is not smooth may contain fine vacuolization such as occurs in the plasmatocyte. In these regions the cytoplasm tends to spread more than in the smooth regions (*D*, bb_{3-4}) and resembles that of certain other cells, for example, the plasmatocytes. The cell periphery is more distinct where the surface is smooth than where the cytoplasm is vacuolized and spread. A cell having vacuolized and spread cytoplasm and the deep chromatic quality of the liocyte is considered to be a liocytoid, although none of its periphery may have the extreme smoothness characteristic of the liocyte. A cell is also classed as a liocytoid when the vacuolization is slight and all but a small part of the periphery is smooth. Nonfusiform cells, s.d. 4.8μ–9.7μ, l.d. 6.4μ–16.1μ; fusiform cells, s.d. 6.4μ–8.1μ, l.d. 10.7μ–18.3μ.

The liocytes and liocytoids graduate one into the other with regard to both size and form. The liocytoids seem to be transitional forms, for example, between the liocytes and the microplasmatocytes, the mesoplasmatocytes, or the oenocytelike cells. Some of the oenocytelike cells resemble the liocytoids very closely.

CLASS III. OENOCYTELIKE CELLS

The oenocytelike cells include the oenocytoids and pseudoenocytoids. Some of the latter may be early developmental forms.

TYPE 5. PSEUDOENOCYTOID

(Oenocytoidlike cell)

The cytoplasm of the pseudoenocytoid ranges from deeply basophilic to amphophilic or slightly eosinophilic. It is usually more homogeneous than that of the plasmatocyte, but occasionally it may exhibit a little coarse, colorless vacuolization or contain one or two gross vacuoles or fine granules. The nuclei are most frequently round but may be ovoid. Usually they have an eccentric location in the cell and seem larger than the nuclei of the oenocytoids. They are variably obscured by cytoplasm, and may appear very dark when the cytoplasm is deeply stained. The periphery of the cell may be distinct or indistinct. The cell is usually round or ovoid but may be somewhat irregular. Compared with the plasmatocyte, it may be thick relative to length and breadth. Nonelongated cells, s.d. 6.5μ–11.8μ, l.d. 7.5μ–22.6μ; elongated cells, s.d. 6.4μ–8.6μ, l.d. 19.3μ–21.5μ.

The pseudoenocytoids are not separated from the oenocytoids, liocytes, liocytoids, or proleucocytes by definite size or form gaps. In general, this type includes all cells that resemble but are not typical oenocytoids and do not fall into the other categories.

TYPE 6. OENOCYTOID

The oenocytoids, so called by Poyarkoff (*24*) and Hollande (*7*), have relatively small, round, grossly punctate nuclei, which frequently occupy an eccentric position in the cell (pl. I, E, cb_{1-3} and cb_{7-9}), and occasionally may be ovoid (cb_4). In most cells the cytoplasm is relatively great in amount and basophilic, but it varies from deeply basophilic to amphophilic or, in a few cells, to slightly eosinophilic. It most frequently has a nearly opaque, homogeneous appearance, which is characteristic of this type of cell. When very intensely basophilic, the cytoplasm may be suggestive of that in the liocyte (cb_{5-6} and cb_{10}). Infrequently it may contain eosinophilic granules. In some oenocytoids the cytoplasm may have a texture suggesting a condition of stress and strain or included crystals or rods not readily visible to the eye. This condition can be observed not only in stained smears (cb_{3-4}), but also in unstained cells moving freely in samples of blood taken from either heat-fixed or normal, living larvae. In other oenocytoids distinct, elongated, eosinophilic inclusions are sometimes present in the cytoplasm (cb_7 and cb_{11}). Occasionally oenocytoids have cytoplasm with a blotched or granular appearance. The nucleus may be more or less obscured in the deeply basophilic cells (cb_1, cb_{5-7}, and cb_{10}) but tends to be distinct in the lighter cells. The cell periphery ranges from very distinct (cb_6) to indistinct (cb_4) but is usually fairly distinct. Oenocytoids may be round (cb_1, cb_{10}), ovoid (cb_2, cb_{4-5}, cb_{7-8}), or polymorphic (cb_3, cb_6, cb_{11}). Some of the polymorphic oenocytoids are more or less pyriform (cb_{2-3}), and some may be very elongated and have either central or eccentric nuclei. The cell may range from thin (cb_2, cb_4, cb_8) to relatively thick (cb_{5-7}, cb_{10}). Nuclear cytoplasmic transfer is sometimes evident (cb_2). Nonelongated oenocytoids, s.d. 5.4μ–14.0μ; l.d. 6.5μ–32.3μ.

CLASS IV. PLASMATOCYTES

(Cell with conspicuous cytoplasm)

The plasmatocytes have different amounts of cytoplasm relative to their nucleus. The cytoplasm has a fine vacuolization, that may be developed to a different extent in the different regions of a single cell or from cell to cell, and may or may not contain one or more gross vacuoles. Most of the plasmatocytes are included in the four types eoplasmatocytes, microplasmatocytes, mesoplasmatocytes, and macroplasmatocytes. These types graduate one into another, and the eoplasmatocytes are linked to the microplasmatocytes through the eoplasmatocytoids. They are identified to some extent through differences in size but largely through differences in relative amount of cytoplasm and degree of vacuolization. The microplasmatocytes, mesoplasmatocytes, and macroplasmatocytes obviously represent successive stages in the development of a single kind of cell. Their relation to the eoplasmatocytes and the eoplasmatocytoids is not entirely clear.

Except possibly the eoplasmatocytes, which have been observed to a less extent in live preparations, the plasmatocytes possess cytoplasm divisible into ectoplasmic and endoplasmic regions. This differentiation may become evident only when the cell assumes its active form. Then the ectoplasm may appear more basophilic than the endoplasm or as a thinner, less intensely stained, hyaloplasmic membrane extended from the cell surface (*35*). The cells in plates II, *A*, *df₄*, and IV, *F*, *d₁*, are plasmatocytes that have transformed partially into their active form. They exhibit a more deeply stained ectoplasm and a less deeply stained endoplasm containing the nucleus.

Macroproleucocytoids and elongated and multiramous plasmatocytes are found rarely.

The imaginal plasmatocytes differ a little from the larval plasmatocytes. Usually they have a more uniform cytoplasmic vacuolization in which the individual vacuoles appear more nearly circular and are more sharply delimited. The imaginal plasmatocytes, particularly the larger ones, appear especially flattened or sheetlike and often are more irregular, folded, or otherwise distorted in a smear (plate II, *A*, *df₉* and *df₁₃₋₁₁*). The following description applies particularly to the larval plasmatocytes.

In general, the plasmatocytes have brightly eosinophilic, punctate nuclei, across some of which a band of cytoplasm is seen (*34*). The nuclei are usually round or ovoid. Several basophilic nucleoli may be visible. The cytoplasmic basophilia varies in different cells from light to deep and tends to decrease from the microplasmatocytes through the mesoplasmatocytes to the macroplasmatocytes. The extent to which the deeply basophilic cytoplasm may obscure the nucleus differs in different cells and sometimes in the same cell. The distinctness of the cell periphery depends largely upon the extent to which the cytoplasm may be spread and to which the cell has undergone passive-active transformation. The gross colorless vacuoles found in these cells correspond to the glycogen inclusions described previously (*34*). The plasmatocytes may be round, ovoid, polymorphic, fusiformoid, or fusiform.

Type 7. Macroproleucocytoid

(Large proleucocytelike cell)

Very infrequently cells are encountered that are too big to be proleuocytes but, like them, contain relatively large round or ovoid nuclei and small amounts of cytoplasm. The nuclei are punctate and resemble those of the plasmatocytes. The cytoplasm is homogeneous or nearly homogeneous, a condition distinguishing the cell from the microplasmatocytes. Some of the eoplasmatocytoids resemble cells of this type.

Type 8. Eoplasmatocyte

(Early plasmatocyte)

The eoplasmatocyte is a round, ovoid, fusiformoid, or fusiform cell having lightly basophilic cytoplasm and a very conspicuous, brightly eosinophilic nucleus (pl. I, *F*, *db₁₋₂*). It appears to be very thin and flat. The cytoplasm usually appears slightly or faintly vacuolized and does not obscure the nucleus unless the cell is overstained. Eoplasmatocytes vary in size but tend to be moderately large (of the order of 11μ by 14μ) with fairly wide cytoplasmic rims. The vacuolization is not so well developed as in the mesoplasmatocytes or the macroplasmatocytes, and the cytoplasmic rims are usually too wide for the cells to be identified as microplasmatocytes.

Type 9. Eoplasmatocytoid

(Cell resembling an eoplasmatocyte)

Some cells appear intermediate between the eoplasmatocytes and the microplasmatocytes. These and other cells, which resemble but are not typical eoplasmatocytes or microplasmatocytes, and do not belong to the other categories, are grouped together as eoplasmatocytoids.

Type 10. Microplasmatocyte

(Cell with poorly developed cytoplasm)

The microplasmatocytes have a small amount of cytoplasm, the fine vacuolization of which tends to be poorly developed. The cells differ greatly in size. The very small cells are not easily distinguished from the larger proleucocytoids.

The larger cells are as big as many of the mesoplasmatocytes. Some of the more basophilic cells, as dd_2 in plate I, G, may resemble liocytoids. The microplasmatocyte tends to have a more hazily stained cytoplasm than either the mesoplasmatocyte or the macroplasmatocyte. The average width of the cytoplasmic rim is nearly always less than half the average diameter of the nucleus, as judged by eye. Nonfusiform cells, s.d. 4.3μ–13.0μ, l.d. 6.4μ–20.5μ; fusiform cells, s.d. 3.2μ–10.8μ, l.d. 9.1μ–32.3μ.

There is no sharp line of distinction, in size or form, between these cells and the proleucocytes, liocytoids, eoplasmatocytes, mesoplasmatocytes, or elongated plasmatocytes. Microplasmatocytes are shown in plates I, B, dd; G, dd_{1-13}; H, dd; II, A, dd_{1-2}; C, dd; III, B, dd_{1-2}; and IV, A, dd; D, dd; F, dd.

TYPE 11. MESOPLASMATOCYTE

(Cell with intermediate cytoplasm)

The mesoplasmatocyte, in general, is intermediate between the microplasmatocyte and the macroplasmatocyte with respect to cytoplasmic development and cell size. The cytoplasm of the mesoplasmatocyte has a more definitely developed fine vacuolization than that of the microplasmatocyte, but the vacuolization is more ragged, more irregular, and more channeled than that of the macroplasmatocyte. The mesoplasmatocytes differ greatly in size. Some of the more chromatic cells are not easily distinguished from the more spread liocytoids, and some of the smallest cells resemble the larger proleucocytoids. The average width of the cytoplasmic rim is usually less, but sometimes greater, than half the average diameter of the nucleus, as judged by eye. Nonfusiform cells, s.d. 5.3μ–16.1μ, l.d. 9.6μ–21.5μ; fusiform cells, s.d. 5.3μ–14.0μ, l.d. 10.7μ–53.8μ.

Mesoplasmatocytes are shown in plates I, A, d_2; H, de_{1-10}; II, A, de_{1-2}; III, A, de; and IV, B, de_{1-2}. The vacuolization of the deeply basophilic cytoplasm in the cells in plate I, H, de_{7-8}, does not show in the illustration.

TYPE 12. MACROPLASMATOCYTE

(Cell with well-developed cytoplasm)

The vacuolization of the macroplasmatocyte tends to be less ragged and irregular than that of the mesoplasmatocyte. The most fully developed vacuolization seems to consist of tiny spaces of about the same size distributed throughout the cytoplasm. The finer vacuolization of the plasmatocyte cannot be seen clearly in the plates, but suggestions of it can be detected in plate II, A, in cells df_1, and df_{3-4}, and especially in df_{7-8}, df_{10}, and df_{13}, in which the vacuolization is not so fine. This cell generally is larger than the mesoplasmatocyte. The average width of the cytoplasmic rim is usually greater than half the diameter of the nucleus as judged by eye. Nonfusiform cells, s.d. 10.7μ–19.4μ, l.d. 11.8μ–25.8μ; fusiform cells, s.d. 7.5μ–17.2μ, l.d. 18.3μ–64.5μ.

Macroplasmatocytes are shown in plates I, C, df; H, df; II, A, df_{1-14}; III, A, df_{1-2}; and IV, B, df (a very large cell, only part of which is shown).

TYPE 13. ELONGATED PLASMATOCYTE

Some fusiform cells that are classed as plasmatocytes because of their nuclear and cytoplasmic structure have very elongated spindle ends with very fine terminations. The width and length of these cells vary from about 3.2μ to 5.9μ and about 22.5μ to 80.6μ, respectively.

No distinct gap separates these cells (pl. II, B, dg) [5] from the ordinary fusiform plasmatocytes (pls. I, C, df; H, de_2; and II, A, de_{1-2}, df_8, df_{11}) or from the nematocytoids (pl. III, A, fa_{1-2}). The plasmatocytes shown in plates I, A, d_1, and II, A, dd_1, represent a borderline condition between the elongated and the more usual fusiform cells.

TYPE 14. MULTIRAMOUS PLASMATOCYTE

Occasionally cells that are classed as plasmatocytes because of cytological characteristics other than form have three instead of two spindle ends (pl. II, C, dh). These and similar cells having more than three spindle ends, which have not yet been observed but might be found in the future, constitute the multiramous plasmatocytes. No definite transitional forms between the triramous plasmatocytes and the triramous polypodocytes (pl. II, D, eb) have been observed,

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[5] The terminations of the very long ends of this cell do not show in the illustration.

although such a transition would seem quite possible. The triramous plasmatocytes and podocytes differ chiefly in cytoplasmic texture. The former have the more compact cytoplasm.

CLASS V. PODOCYTES

(Cell with footlike extensions)

The podocytes include the propolypodocytes and the polypodocytes. The cytoplasm is lightly basophilic and does not obscure the nucleus. It has a looser or more irregular type of vacuolization than the macroplasmatocyte and may or may not contain one or more gross, colorless vacuoles. These vacuoles probably represent glycogen inclusions (*34*). The nuclei of these cells are very punctate and brightly eosinophilic, especially in the more flattened polypodocytes having more than three rami.

The podocytes are very thin, flat cells. Their cytoplasmic extensions characterize the passive form and apparently do not indicate pseudopodial locomotion, although pseudopodial extensions do occur when the cell is in its active state.

TYPE 15. PROPOLYPODOCYTE

(Early polypodocyte)

The propolypodocytes (pl. II, *D, ea*) are fusiform cells, which are apparently transitional forms between the fusiform plasmatocytes and the polypodocytes. Frequently they are larger than most of the mesoplasmatocytes and some of the macroplasmatocytes. Their spindle ends are usually long, and they frequently have, in addition, one or more shorter extensions of the cytoplasm.

TYPE 16. POLYPODOCYTE

(Cell with many footlike extensions)

The polypodocytes (pls. I, *C, eb;* II, *D, eb;* III, *A, eb*) have round or nearly round nuclei. The pseudopodlike extensions of the cytoplasm are usually long, tapering from the main body of the cell until their distal ends become extremely fine, and in many instances are practically invisible (oil immersion, 1500×). Vacuolization occurs both in the main body of the cytoplasm and in the large proximal portions of the extensions. The cell periphery thus varies from distinct to almost invisible. Like the cell body, the cytoplasmic extensions are flattened, noticeably in their proximal regions. In spite of its extensions the polypodocyte is fundamentally a fusiform cell, for frequently two of its cytoplasmic extensions are a little larger or longer than the others (pl. I, *C, eb*). The width of the cell body not including extensions is $7.5\mu–12.0\mu$; the cell length, $35.5\mu–40.5\mu$.

No size or form interval distinguishes the polypodocytes from the propolypodocytes.

CLASS VI. VERMIFORM CELL

(Wormlike cell)

The vermiform cells are extremely elongated fusiform cells. They include the nematocytoids. The nematocytoids are transitional forms between the elongated plasmatocytes and the nematocytes. Vermiform cells are rarely triramous. Their nuclei are elongated, ovoid, and punctate, and may be banded by cytoplasm. The cytoplasm is basophilic, usually lightly so, and is slightly vacuolized, particularly in the perinuclear region. It may or may not contain one or more gross vacuoles, which are likely to be glycogen inclusions (*34*). The long spindle ends may become very fine and indistinct at their extremities (oil immersion, 1500×). The cells are somewhat flattened.

No size or form interval distinguishes the nematocytoids from the nematocytes or from the elongated plasmatocytes. Plate III, *A, fa₁*, shows a cell just beginning to transform from an elongated plasmatocyte into a nematocytoid.

TYPE 17. NEMATOCYTOID

(Cell resembling nematocyte)

The nematocytoids (pl. III, *A, fa*₁₋₂) are elongated more than the elongated plasmatocytes but less than the nematocytes. They vary in size, but may be of the order 4.5μ by 75μ.

Type 18. Nematocyte

(Threadlike cell)

The nematocytes (pl. III, *A, fb*) are the longest cells found in the blood stream of the southern armyworm. Their dimensions vary. They may attain a length of over 300μ and, at the same time, have a width as small as 2.5μ in the region of the nucleus. Although these cells may appear threadlike, they are essentially fusiform cells and are slightly flattened.

Type 19. Multiramous Vermiform Cell

Nematocytes or nematocytoids rarely have been found with three instead of two spindle ends and probably could possess more. These are considered to be multiramous vermiform cells.

CLASS VII. CYSTOCYTES

(Cell with cystlike inclusions)

The cytoplasm of the cystocytes (pl. III, *A, gc; B, gc_{1-9}*) contains eosinophilic matter in the form of inclusions, referred to here as "cysts."[6] The degree of eosinophilia ranges from slight (very pale orange) to intense (bright orange-red), although infrequently the cysts are colorless or nearly so. There is also wide variation in the number of inclusions within a single cell. The cysts vary in size and may be round, ovoid, or irregular. The cytoplasm itself is basophilic, and occasionally seems to contain basophilic granules, which might be only the intercystic cytoplasmic strands seen in optical section. These cells may be round, ovoid, polymorphic, fusiformoid, or fusiform. In the fusiform cystocyte the eosinophilic inclusions, the nucleus, and the greater part of the cytoplasm are confined to the main body of the cell, giving the cell body a bulky aspect (pl. III, *A, gc; B, gc_2 and gc_9*). The round, ovoid, or polymorphic cell may exhibit a definite ectoplasmic region, depending upon whether or not it has transformed into the active state. The nuclei are eosinophilic but usually of a less brilliant, more nearly opaque, red color than the intensely eosinophilic cysts. The nuclei are punctate, are nearly round to ovoid, may appear very flattened, and may be eccentrically located in the cells. They may be banded, but the banding is usually obscured by overlying cytoplasmic structures. In some cells the nuclei seem basophilic, possibly because the cytoplasm immediately surrounding them is deeply basophilic. (The nuclei may appear blue in smears not properly differentiated.) The cell periphery varies from very distinct to indistinct. The fully developed cystocytes are usually the most conspicuous because of their large size and red color.

Type 20. Cystocytoid

(Cell resembling a cystocyte)

The cystocytoids are fusiform cells with clear spindle ends and coarse, irregular vacuolization in the perinuclear cytoplasm. The cytoplasm may contain one or more cysts, which may be colorless or may exhibit very faint indications of eosinophilia. The cytoplasm tends to be darkly basophilic. It may or may not contain one or more gross colorless vacuoles, which are apparently glycogen inclusions. The nucleus is punctate and eosinophilic but may be darkened and obscured by overlying cytoplasm. The cell contour may vary from distinct to indistinct. The nucleus may occasionally be banded by cytoplasm.

These cells occur very infrequently. Like the plasmatocytes and the cystocytes, they may become rounded. They seem to be occasional transitional forms between the plasmatocytes and the neocystocytes. The plasmatocyte in plate I, *H, de_{10}*, resembles a cystocytoid but contains no cysts.

Type 21. Neocystocyte

(New cystocyte)

The neocystocytes (pl. III, *B, gb*) contain partially but variably developed eosinophilia. They may contain many cysts that are only slightly eosinophilic or a few cysts that are intensely eosinophilic. Otherwise they are typical cystocytes.

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[6] The term "cyst" is used for convenience and does not imply that the eosinophilic inclusions have limiting membranes more definite than those of other vacuolar inclusions referred to in connection with other cell types.

No definite size or form interval separates the neocystocytes from the cysto-cytoids, paleocystocytes, or cystocytic plasmatocytes.

TYPE 22. PALEOCYSTOCYTE

(Old cystocyte)

The paleocystocytes exhibit a well-developed eosinophilia (pl. III, A, gc; B, gc_{1-6}, gc_9). The eosinophilic matter may become so plentiful that the main part of the cell body appears bloated (A, gc; B, gc_{1-2} and gc_9). The nuclei may be hidden by the cytoplasmic structure, but usually they are visible. Nonfusiform cells, s.d. 9.7μ–23.7μ, l.d. 12.9μ–26.9μ; fusiform cells, s.d. 6.4μ–18.3μ, l.d. 18.3μ–59.1μ.

No size of form interval separates these cells from the neocystocytes or cysto-cytic plasmatocytes.

TYPE 23. CYSTOCYTIC PLASMATOCYTE

The cystocytic plasmatocytes (pl. III, B, gd) are typical plasmatocytes but contain eosinophilic cysts. They have partially developed eosinophilia, as do the neocystocytes, but lack the clear spindle ends typical of cystocytes.

CLASS VIII. SPHEROIDOCYTES

(Cell with spherical inclusions)

The spheroidoctyes are small cells that contain colorless vacuoles, here called spheroids. The cell outlines tend to be round. No fusiform spheroidocytes have been observed. Spheroidocytes are shown in plates I, C, h; II, A, hb_{1-2}; C, hb; III, A, hb; B, hb; C, ha_{1-8}; and IV, A, hb_{1-9}; B, hc_{1-13}; C, hd.

Occasionally, especially during metamorphosis, spheroidocytes may contain eosinophilic inclusions or they may contain typical spheroids that show some degree of eosinophilia (pl. III, C, ha_{5-6}; pl. IV, B, hc_{11-13}), particularly in smears deeply stained with eosin.

TYPE 24. SPHEROIDOCYTOID

(Cell resembling a spheroidocyte)

The spheroidocytoids from the early instars contain more cytoplasm and fewer spheroids than do the other spheroidocytes. They are apparently early develop-mental forms. The vacuoles of those shown in plate III, C, ha_{1-8}, are nearly lost in the illustration, but some can be detected as faint areas in the blue cytoplasm of cells ha_{1-4} and ha_7, and as more distinct, slightly reddish areas in cells ha_{5-6}. Single cells may contain only one or several spheroids. The cytoplasm may tend to obscure the nucleus, which may be slightly eccentric. The periphery of the cell may be distinct or indistinct. The cell is small. It is usually round to slightly ovoid, but may be somewhat irregular. S.d. 4.3μ–9.7μ, l.d. 4.3μ–14.0μ.

Spheroidocytelike cells that do not fall into other classes or other types of spheroidocytes and are encountered especially in the later instars, pupa, and adult, are classified as spheroidocytoids, although they might be transitional forms.

No size or form interval separates the cells of this type from the orthospheroido-cytes.

TYPE 25. ORTHOSPHEROIDOCYTE

(Typical spheroidocyte)

The orthospheroidocytes are typical of the late instars up to about the beginning of the prepupal stage. The cells may be round, slightly ovoid, or otherwise somewhat irregular in outline. Their nuclei are round, slightly ovoid, or slightly anguloid; they are also punctate, have large chromatin masses relative to nuclear size, and may be variably obscured by overlying cytoplasm. The nucleus and cell frequently look slightly angular. The cytoplasm appears as irregular baso-philic areas separating the spheroids. These spheroids differ in size, shape, and distinctness and fill the cytoplasm to various degrees, giving the cell periphery an irregular and indistinct aspect. In some smears many of these cells contain crystals. S.d. 4.8μ–9.7μ, l.d. 5.4μ–10.8μ.

Orthospheroidocytes are shown in plates II, A, hb_{1-2}; C, hb; III, B, hb; and IV, A, hb_{1-9}. Although the details of the more lightly stained cells in the group at hb_1 in plate IV are faint in the illustration, the cells show numerous spheroids as light areas in the lightly stained cytoplasm. The cell at the left of and a little above the label hb_1 contains an angular crystal below the nucleus.

Type 26. Metaspheroidocyte

(Altered or intermediate spheroidocyte)

The metaspheroidocytes (pl. IV, B, hc_{1-13}) are characteristic of the prepupal, particularly late prepupal, and the pupal stages. They tend to be larger and thicker than the orthospheroidocytes, to contain greater numbers of spheroids, and to be more evenly rounded unless they have begun to degenerate. The nuclei frequently appear relatively smaller than those of the orthospheroidocytes as they lie in the enlarged cytoplasm, and they are often very eccentrically located (hc_6, hc_8, hc_{10}, and hc_{12}). Frequently the spheroids seem larger and more distinct than those of the orthospheroidocytes. The cell contour may appear broken in places, especially after degeneration has begun (hc_{1-4}). S.d. $6.4\mu–12.9\mu$, l.d. $8.6\mu–18.3\mu$.

The metaspheroidocytes appear to be orthospheroidocytes that have become hypertrophied and otherwise altered.

Sometimes metaspheroidocytes are partially eosinophilic or contain eosinophilic inclusions, and consequently appear more or less rhegmatocytic (pl. IV, B, hc_{11-13}). They might, but probably do not, represent transitional forms between the spheroidocytes and the rhegmatocytes. Metaspheroidocytes frequently contain basophilic or eosinophilic inclusions that appear to have been phagocytized (hc_5).

Type 27. Paraspheroidocyte

(Nearly like the orthospheroidocyte)

The paraspheroidocytes are found in the adult. The lightly basophilic cytoplasm has a vacuolization resembling but not identical with that of the orthospheroidocytes. A paraspheroidocyte with a slightly eccentric nucleus is shown in plate IV, C, hd. The nucleus sometimes has a very eccentric location, giving the cell an appearance more or less like that in B, hc_6. Some cells have a turgid aspect. Paraspheroidocytes usually have a round to ovoid outline but may be somewhat irregular.

CLASS IX. ERUPTIVE CELLS

The eruptive cells consist of the rhegmatocytes and rhegmatocytoids. In contrast to the bright eosinophilia of the nuclei in the plasmatocytes and polypodocytes and of the eosinophilic cysts, the eosinophilia of the eruptive cells is relatively dull and nearly opaque, even when intense.

Type 28. Rhegmatocytoid

(Cell resembling a rhegmatocyte)

The rhegmatocytoids can be classed as intact and erupting or erupted. These cells differ greatly in appearance. The intact cells (pl. I, F, ia and III, C, ia) have fairly distinct to indistinct nuclei and some visibly basophilic cytoplasm, usually very small in amount but more abundant in some of the developmental forms found in the early instars. The cytoplasm contains one or more intensely eosinophilic vesicles and sometimes one or more colorless vacuoles, here called colorless vesicles. Many of the rhegmatocytoids of the early instars (about the third) are very vesicular (pl. IV, D, ia_{1-2}). Most of the intact rhegmatocytoids are small cells.

The erupted or erupting rhegmatocytoids (pl. IV, D, ia_3) may exhibit a general or a localized eosinophilia, apparently caused by eosinophilic matter from the cell, which may affect the adjacent plasma. The cytoplasm may contain one or more intensely eosinophilic vesicles, usually contains none to several colorless vesicles, and may have granules that are eosinophilic to different degrees. The nuclei and the nuclear-cytoplasmic color differentiation may be partly or completely obscured. The cell periphery may vary from fairly smooth to ragged and from fairly distinct to indistinct. S.d. $5.4\mu–10.8\mu$, l.d. $5.4\mu–12.9\mu$.

Occasionally cells resemble spheroidocytes but have the dull eosinophilia of the rhegmatocytoids (pl. IV, B, hc_{11-13}). Such cells are classed according to the type they most closely resemble.

The rhegmatocytoids, especially of the earlier instars, are presumptive precursors of the rhegmatocytes or are transitionals between the rhegmatoyctes and some other type.

Type 29. Rhegmatocyte

(Cell that erupts)

For purposes of description rhegmatocytes can be classified as leucovesicular, eosinovesicular, erupting, and erupted, although no sharp line of demarcation separates them. They are very unstable cells containing cytoplasmic vesicles and granules. Under certain conditions they erupt and cast some of the material from the vesicles into the surrounding plasma.

The cytoplasm of the leucovesicular rhegmatocytes may show some basophilia and contain one to several, usually large, colorless vesicles, one to several intensely eosinophilic vesicles, and some intervesicular, eosinophilic granules. The cell periphery may vary from distinct to indistinct, may be irregular, and sometimes may appear more or less granular. The leucovesicular rhegmatocytes in general resemble the rhegmatocytoids in pl. IV, D, ia_{1-2}, but they usually exhibit less basophilic cytoplasm.

The eosinovesicular rhegmatocytes (pl. IV, B, ib_{1-2}, and E, ib_3) contain no colorless vesicles. Their cytoplasm usually appears eosinophilic and contains no colorless vacuolization. The intact rhegmatocytes (B, ib_{1-2}, and E, ib_{3-4}) contain one or more, frequently several, eosinophilic vesicles that appear less blue and more red than do those in the illustration. Nuclear-cytoplasmic differentiation may be entirely obscured by the eosinophilia that invests the entire cell. The cell periphery is usually distinct. The cell outline tends to be smoothly irregular. Frequently the cell is fairly thick and mulberry-shaped, especially in drops of unstained blood from living larvae.

The erupting rhegmatocytes [7] possess cytoplasm with little or no detectable basophilia, most of the regions appearing eosinophilic. The cytoplasm may contain none to several eosinophilic and colorless vesicles and some eosinophilic granules. The nucleus is eosinophilic. The plasma adjacent to a cell may contain eosinophilic erupted matter appearing as granules, larger globules, or both. Nuclear-cytoplasmic color differentiation is usually obscured. The cell periphery is fairly distinct to indistinct, tending to be obscured. In plate IV, E, ib_4 is an imaginal rhegmatocyte beginning to erupt, and ib_5 is a partially erupted one.

The erupted rhegmatocytes apparently are the remains of cells after a complete eruption that probably takes place while the insect is being heat-fixed (pl. II, A, ib_1; IV, E, ib_6). The nuclei, when visible, are somewhat eosinophilic and are fairly distinct to indistinct, most frequently the latter. The cytoplasm may show a very little basophilia but usually is slightly eosinophilic. Frequently it appears as an irregular remnant about the nucleus, is relatively inconspicuous, and may give faint indications of containing one or more emptied vesicles (pl. II, A, ib_1). There is little or no nuclear-cytoplasmic differentiation. The cell periphery usually appears indistinct and irregular or ragged. S.d. 4.3μ–11.8μ, l.d. 5.4μ–17.2μ.

CLASS X. DEGENERATING CELLS

No categorical description of degenerating cells can be given, because they do not comprise a true class or type. They are treated as a class only for convenience and because they cannot be ignored in quantitative differential counts. They include achromophiles and hyaline cells, both of which are questionable types, and degenerating cells of various kinds.

Type 30. Achromophile

(Faintly stained cell)

The achromophile has a faded, degenerated aspect. The nucleus is eosinophilic and sometimes stains more deeply centrally than peripherally. The cytoplasm is basophilic, usually lightly stained, and very faintly and raggedly vacuolized. The cell contour is faintly visible to invisible. The cell is suggestive of a thinly spread, active form. It is probably a degenerative form.

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[7] In blood freshly drawn from a living larva the rhegmatocytes have a mulberry shape, apparently because of the large vesicles in the cytoplasm. As the cells are moved by currents of the plasma, some can be seen to erupt. Material that seems to come from one or more of the vesicles passes through limited regions of the cell surface into the plasma, where it appears as small droplets. As the cells are carried on by the currents in the plasma, the erupted droplets are left behind and gradually increase in size, probably taking up material (water?) from the plasma. After erupting, the cells exhibit vacuolar spaces in the cytoplasm, which no doubt were previously occupied by the erupted matter. A partially erupted cell may at the same time contain both unerupted and empty vesicles.

Type 31. Hyaline Cell

The hyaline cell has a variably faint, eosinophilic nucleus and a slightly eosinophilic (pale orange or orange) cytoplasm that is hyaline or nearly hyaline and faintly visible. The periphery is smooth or nearly smooth. A cell outline varies from round to fusiform, and may be irregular. It is probably a degenerative form.

Type 32. Degenerating Cells

Those hemocytes that are so degenerated that they cannot be identified with certainty are classified as degenerating cells.

DIFFERENTIAL COUNTS

The differential counts for the entire life cycle of the southern armyworm are given in table 2. The count from the 50.5-hour-old fifth instar is the mean of five independent counts made from a single hemolymph smear. The five counts, their mean, and the differences between the mean and the high and low values are shown in table 3.

TABLE 2.—*Differential counts of the blood cells of the southern armyworm*

Stadium	Age within stadium	Counts	I	II	III	IV	V	VI	VII	VIII	IX	X	Average cells counted	Mitotic figures
	Hours	*Number*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Number*	*Percent*
First	43.5–44.0	5	21.3	18.2	1.3	46.8	0	0	0	2.5	8.9	0.4	134	0.7
Second	5–1.5	4	19.7	5.9	.9	50.0	0	0	0	2.5	18.9	.9	198	1.2
	25–39	3	4.6	12.4	5.3	40.0	0	0	0	11.2	21.4	2.4	183	1.7
Third	.3	2	10.6	6.4	2.4	33.0	0	0	0	3.5	43.4	.2	338	.5
	24.0	2	9.2	11.3	2.1	44.7	0	0	0	2.2	23.4	6.9	212	0
	49.0–49.5	1	12.8	9.6	2.0	31.3	0	0	0	12.4	31.6	0	451	.2
Fourth	1.5–3.0	1	.6	5.3	2.2	33.6	0	0	0	13.8	39.0	4.1	317	.9
	27.5–42.0	1	1.6	19.6	3.8	48.2	0	0	.8	5.5	18.0	1.6	337	.8
	(1)	1	.5	27.6	1.0	45.1	0	0	0	13.1	12.7	0	221	0
Fifth	.5	1	4.1	20.5	5.1	39.0	1.4	0	.7	16.1	11.3	1.0	292	.7
	0–21	1	1.6	25.8	1.3	34.3	0	0	.3	14.3	19.7	.3	310	1.0
	25.8	1	.3	7.3	1.2	12.0	0	0	6.9	24.2	47.1	.3	318	.6
	29.5	1	2.7	7.8	3.6	40.7	.6	0	2.1	29.6	11.0	1.5	334	.3
	50.5	(2)	.05	6.9	3.1	41.8	.2	0	10.0	22.9	14.3	.05	400	.3
	84.0	1	1.5	5.6	0	67.3	.8	0	7.2	6.6	10.5	0	267	1.1
Sixth	(3)	1	5.5	10.9	6.3	24.3	0	0	4.6	16.1	30.7	.5	366	1.1
	26	1	4.3	2.3	6.6	41.7	.7	0	11.8	16.5	15.6	0	442	.5
	75.5	1	2.4	2.6	2.2	46.4	.2	0	8.5	9.6	25.7	1.7	459	.7
	152	1	0	2.8	8.3	35.9	1.5	0	4.5	31.4	15.0	0	598	.2
	(4)	1	2.2	1.7	6.5	55.8	.6	0	1.7	21.4	8.9	.2	542	.7
Prepupa	(5)	1	2.3	1.9	5.1	37.5	.9	.2	5.2	38.1	8.5	0	422	0.2
	(6)	1	2.2	1.2	8.3	37.5	0	0	5.5	33.9	3.6	7.4	419	
	(7)	1	.4	2.5	1.8	14.9	1.1	.2	28.0	43.9	6.0	.7	436	.7
Pupa, male	0–.5	2	3.1	3.4	5.4	28.6	.7	0	13.1	24.4	20.4	.1	413	.4
	20.75	1	1.2	5.0	.7	48.7	0	0	7.5	26.0	10.7	.5	400	.2
	71.8–79.0	2	4.1	1.5	0	37.3	0	0	19.4	0	4.3	33.3	400	.2
	101.8–102.5	2	15.9	1.6	0	42.3	0	0	13.8	0	0	26.6	212	.1
Pupa, female	.5	1	1.7	1.7	.5	33.7	.7	0	15.7	27.7	1.2	16.2	400	0
	5.8	1	1.0	5.5	0	26.5	.2	4.5	45.6	15.5	0	1.0	400	0
	14.3–14.8	2	1.2	1.5	.1	41.6	1.0	.2	23.0	28.0	.4	2.6	400	.1
	20.8	1	3.5	1.0	.2	73.0	0	0	12.0	.2	1.0	8.2	400	.7
	95.3	2	11.4	.1	0	46.7	0	0	3.7	.5	.6	36.1	279	0
	102.5	2	6.3	0	0	36.1	0	0	11.9	2.1	0	42.4	400	.3
	147	1	2.7	0	0	19.7	0	0	4.0	0	19.5	56.2	400	.2
Adult, male	.12	1	7.0	.7	0	59.5	0	0	0	0	0	32.7	400	0
	.12–16.5	1	4.5	.7	0	77.0	0	0	0	0	0	17.5	400	0
	47.5	2	5.1	.2	0	89.7	0	0	0	.9	0	3.6	400	.4
	90–106.5	3	3.0	.5	0	84.2	0	0	0	.1	.1	11.6	364	.1
	144	1	7.9	0	0	80.9	0	0	0	0	0	11.1	63	0
	151	1	4.4	0	0	79.7	0	0	0	0	0	15.7	267	0

TABLE 2.—*Differential counts of the blood cells of the southern armyworm*—Con.

Stadium	Age within stadium	Counts	Cell class										Average cells counted	Mitotic figures
			I	II	III	IV	V	VI	VII	VIII	IX	X		
	Hours	*Number*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Num-ber*	*Per-cent*
Adult, female.	0.0–0.4		6.2	0.5	0	65.2	0	0	0	1.3	1.6	24.5	400	0.2
	.5–16.5	1	2.7	2.7	0	89.0	0	0	0	1.7	1.2	2.2	400	.2
	18.3–19.8	2	4.6	2.0	0	80.6	0	0	0	2.0	.2	10.3	400	.1
	47.8–50.0	2	1.0	.4	0	80.2	0	0	0	.4	.1	17.7	400	.1
	70.3	1	1.2	.2	0	79.0	0	0	0	3.5	6.0	10.0	400	.5
	77.0–93.5	4	1.2	0	0	85.6	0	0	0	1.0	6.2	5.3	400	0
	117	1	.7	0	0	78.0	0	0	0	0	17.3	3.7	400	0
	151	1	0	0	0	62.7	0	0	0	0	.7	37.5	400	0
	185	1	0	0	0	82.3	0	.0	0	0	3.0	14.0	400	.7

[1] Just prior to molt.
[2] Mean of 5 counts made on a single smear.
[3] During molt.
[4] Away from food.
[5] Just entering ground.
[6] Beginning to taper.
[7] Well tapered.

TABLE 3.—*Repetitions of a differential count of the blood cells of a 50.5-hour-old fifth instar of the southern armyworm*

Count No.	Cell class										Mitotic figures
	I	II	III	IV	V	VI	VII	VIII	IX	X	
	Per-cent	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*
1	0	9.25	2.75	48.00	0	0	10.00	16.50	12.75	0	0.50
2	.25	6.00	3.00	41.75	.50	0	12.25	23.00	13.00	.25	.25
3	0	6.25	3.25	31.25	.25	0	9.00	31.25	18.25	0	.50
4	0	5.25	3.50	42.00	.25	0	8.50	22.50	17.75	0	.25
5	0	8.00	3.25	46.25	.25	0	10.25	21.50	10.00	0	.25
Mean	.05	6.95	3.15	41.85	.25	0	10.00	22.90	14.35	.05	.35
Difference +	.20	2.30	.35	6.15	.25	0	2.25	8.35	3.90	.20	.15
Difference −	.05	1.70	.75	10.60	.25	0	1.50	6.40	4.35	.05	.10

MITOSIS

Reproduction of hemocytes by mitosis occurs in the circulating hemolymph during all stages of development (fig. 2). Although no special study of hemocyte reproduction was made, the mitotic figures observed in making differential counts were noted. Mitotic figures were found among the spheroidocytoids, liocytes (=proleucocytes ?), eoplasmatocytes, pseudoenocytoids, liocytoids, cystocytes, mesoplasmatocytes, microplasmatocytes, orthospheroidocytes, proleucocytes, metaspheroidocytes, and rhegmatocytoids. Most of the mitotic figures were observed among the plasmatocytes, smooth-contour chromophiles, and spheroidocytes.

All stages of mitosis were seen. The prophase was the most difficult to identify, because sometimes, especially near a molt, nuclei appeared generally more ragged and prophasic than at other times, possibly because of very early passive-active changes. A metaphase (l_1) and a telophase (l_2) from a fifth instar are shown in plate IV, *F*.

Hemocytes in a condition suggestive of amitosis were observed very rarely. Occasionally binucleated cells were found. There was no definite evidence that the hemocytes might develop from the cells of other tissues.

651340—45——2
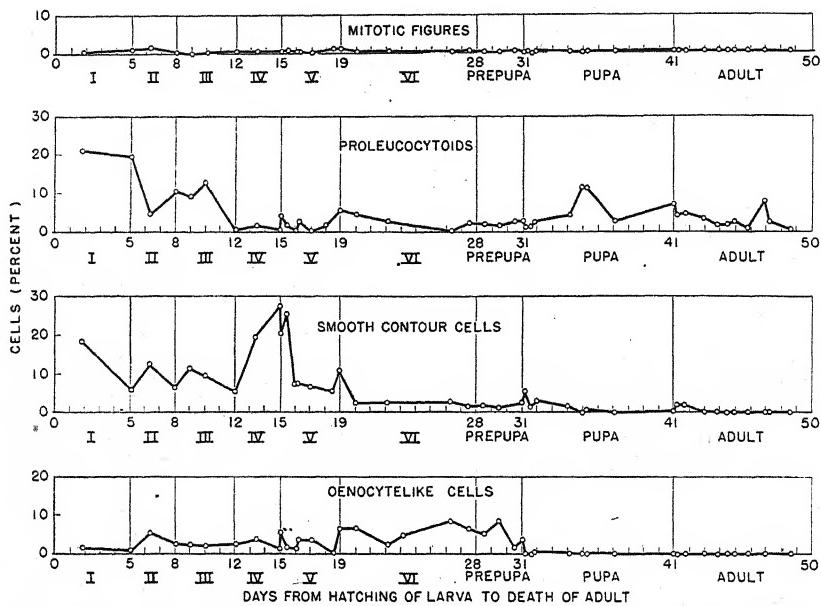
FIGURE 2.—Variation of mitotic figures, proleucocytoids, smooth-contour chromophiles, and oenocytelike cells during the life cycle of *Prodenia eridania*. Roman numerals on abscissa indicate stadia, arabic numerals days after hatching, and vertical lines the times of molting, pupation, and emergence.

RELATIVE VARIATIONS OF HEMOCYTES AT DIFFERENT STAGES OF THE LIFE CYCLE

The relative variations of the different types of hemocytes at different stages in the development of the southern armyworm are shown graphically in figures 2 to 5.
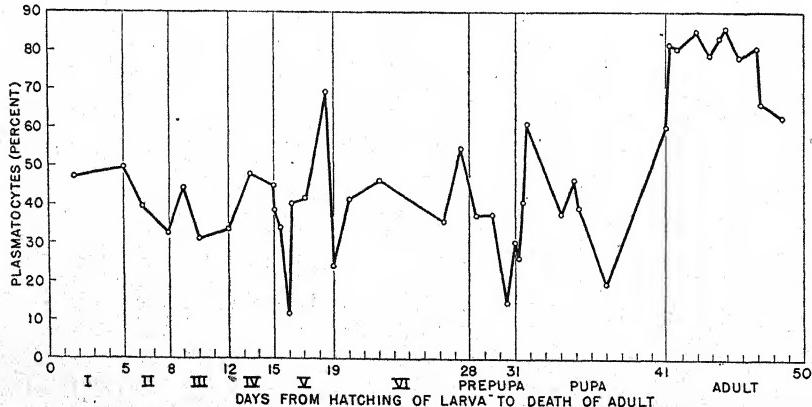

FIGURE 3.—Variation of plasmatocytes during the life cycle of *Prodenia eridania*.

PROLEUCOCYTOIDS (CLASS I)

The proleucocytoids (fig. 2) were present in all stages of the life span. In general they constituted only a small percentage of the hemocyte population. The percentage was relatively high during
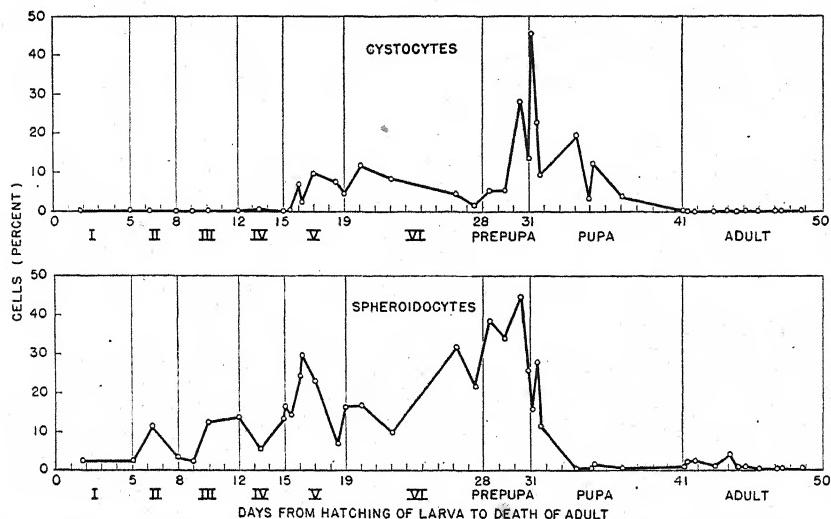
FIGURE 4.—Variation of cystocytes and spheroidocytes during the life cycle of *Prodenia eridania.*
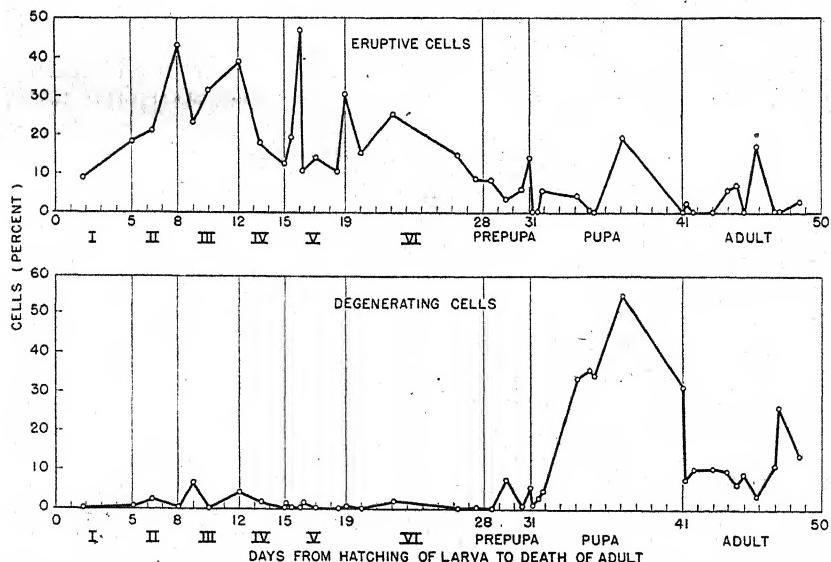
FIGURE 5.—Variation of eruptive cells and degenerating cells during the life cycle of *Prodenia eridania.*

the early stadia and the pupal and early imaginal stages, and a slight rise occurred during the early part of the sixth stadium. The microcytes accounted for only a small proportion of the proleucocytoids. In none of the differential counts did the microcytes rise above 2.8 percent. The fluctuations of the curve therefore largely represent changes in the proleucocytes. Only a few of the proleucocytes were circular, approximately 4 percent in the first stadium and in other stages not more than 1.3 percent of the total hemocytes. In about 40 percent of the counts no round proleucocytes were observed at all.

The average fusiform-nonfusiform (F/N) ratio of the microcytes was 0.0002, and of the proleucocytes 0.01.

SMOOTH-CONTOUR CHROMOPHILIC CELLS (CLASS II)

The smooth-contour chromophiles usually constituted a small percentage of the hemocyte population, but were present in all stages of development. They were most numerous in the first five stadia. Their average F/N ratio was 0.0001. In general, the liocytes and the liocytoids were about equally numerous in the first four stadia, increasing and decreasing in similar manner, but after the beginning of the fifth stadium the liocytoids tended to exceed the liocytes. Both types attained a maximum percentage near the time of the molt from the fourth to the fifth instar, and a minimum late in the pupal stage. The liocytoids increased slightly early in the imaginal stage.

OENOCYTELIKE CELLS (CLASS III)

The oenocytelike cells were present from the first stadium to the first few days of the pupal stage; therefore they occurred rarely, if at all, in the imaginal stage where they were not observed. They never amounted to more than about 9 percent of the hemocyte population and attained a maximum late in the sixth stadium or in the prepupal stage. In the first two or three stadia the pseudoenocytoids exceeded the oenocytoids; in the fourth and fifth stadia the pseudoenocytoids tended to be slightly more numerous, and from that time to the early part of the pupal stage the oenocytoids were more prevalent.

PLASMATOCYTES (CLASS IV)

The plasmatocytes were present in all stages of the insect's development (fig. 3). They fluctuated widely about the 40-percent level until about the middle of the pupal stage. They varied around 80 percent in the imaginal stage, but decreased near the end.

The fall in percentage of plasmatocytes in the well-tapered prepupa occurred at about the time the percentage of spheroidocytes attained a maximum and that of the eosinophilic cystocytes was rapidly rising to a maximum (fig. 4). The low percentage of plasmatocytes in the 147-hour-old pupa was associated with an increase of cells suggestive of either eosinophilic metaspheroidocytes (pl. IV, *B*, hc_{11-13}) or possible eruptive cells, but classified as the latter (fig. 5), and with the very marked rise of degenerative cells to a maximum percentage. The very high percentage of plasmatocytes during imaginal life was associated with the absence of oenocytoids, podocytes, vermiform cells, and cystocytes, and with low percentages of other cell types. The decrease in the percentage of plasmatocytes at the end of imaginal life was accompanied by a corresponding increase in degenerating cells.

The average F/N ratio of the plasmatocytes was 0.53.

The eoplasmatocytes and eoplasmatocytoids were found almost exclusively during the first four stadia. The percentages gradually declined from a maximum in the first stadium to zero in the fourth or early part of the fifth. The fluctuations of the eoplasmatocytoids were large and such that the percentages of these cells were high at the molts but low between molts.

The microplasmatocytes were present in all stages. During the first four stadia they fluctuated inversely with the eoplasmatocy-

toids.   Thus the percentage of microplasmatocytes was low at the molts and higher between molts, particularly in the fourth stadium. The trend of the microplasmatocytes was slightly downward during larval life, except perhaps in the first two stadia.   At about the beginning of the prepupal stage the percentage trended upward, with fluctuations, until in the imago it fluctuated about the 50-percent level but decreased in the last part of this stage.

The mesoplasmatocytes were found in all stages except during the first stadium.   Up to the fourth stadium the percentage was lower at about the time of each molt, fluctuating directly with the microplasmatocytes and inversely with the eoplasmatocytoids.   During larval development these cells trended upward to about the 30-percent level in the latter part of the fifth and the early part of the sixth stadium.   Then they trended gradually downward to only 2 or 3 percent at about the 147th hour of pupal life, meanwhile showing some marked fluctuations, particularly at the beginning of the sixth stadium and at pupation.   During the imaginal stage the mesoplasmatocytes rapidly increased to approximately the 30-percent level, about which they fluctuated widely.

The macroplasmatocytes were present from about the beginning of the fifth stadium to the end of adult life.   They trended upward to about 18 percent in the latter half of the sixth stadium and downward to zero at about the fourth day of the pupal stage.   During the imaginal stage they fluctuated about the 2- or 3-percent level and did not rise higher than about 6 percent.   Sudden decreases of macroplasmatocytes seemed to occur at the time of the molt to the sixth instar and in the latter part of the prepupal stage.

The macroproleucocytoids and the multiramous plasmatocytes were of irregular and very infrequent occurrence.   The elongated plasmatocytes also appeared irregularly, but most frequently during the prepupal and pupal stages, occurring more readily in the female than in the male.   They never amounted to more than 3 or 4 percent of the hemocyte population and usually varied about much lower levels.

PODOCYTES (CLASS V)

The podocytes were observed rarely or not at all before the fifth stadium and were not present in the imaginal stage.   They were found chiefly from the fifth larval to the early part of the pupal stage.   They did not amount to more than 2 percent of the hemocyte population.

VERMIFORM CELLS (CLASS VI)

The vermiform cells appeared infrequently and in variable numbers prior to the fifth stadium, and never in the imaginal stage.   They were present chiefly in the sixth larval, the prepupal, and the very early part of the pupal stage, and seemed to occur in the female pupae more readily than in the male.   In hemolymph smears from some insects they were very conspicuous, though relatively not numerous, but in most smears they were very scarce or apparently absent.

CYSTOCYTES (CLASS VII)

The cystocytes were present from the fourth larval to the latter part of the pupal stage (fig. 4), with a few occasionally in the third stadium.   In the fifth stadium they rose rapidly to about 10 percent,

but during the sixth they gradually decreased. In the prepupal stage they increased rapidly and reached a maximum of about 45 percent at the beginning of the pupal stage; then the percentage dropped gradually to zero. The decrease of cystocytes during the pupal stage was associated with an increase of degenerating cells. The average F/N ratio was 0.34.

The cystocytoids were very scarce and occurred very irregularly. The percentage was never higher than 1.

The neocystocytes were present in the fourth to sixth larval stages and in the prepupal stage. They attained their maximum of about 3 percent during the latter part of the fifth and the first part of the sixth stadium.

The paleocystocytes were found from the fourth larval to the pupal stage. At the beginning of the fifth stadium they rose rapidly to about 6 percent, fluctuated about this level to the end of the sixth stadium, and then decreased. The form of the curve showing percentage of paleocystocytes during the prepupal and pupal stages is essentially the same as that for cystocytes in figure 4.

The cystocytic plasmatocytes appeared chiefly during the prepupal and pupal stages. They rose to about 4 or 5 percent in the first half of the pupal stage, but the percentage never exceeded 6 and usually was less than 2.

SPHEROIDOCYTES (CLASS VIII)

The spheroidocytes were present in all stages (fig. 4). During larval development they gradually trended upward until they attained their maximum of over 40 percent just prior to pupation, and then rapidly decreased to near zero at about the fourth or fifth day of pupal life. Their percentage was low during the first half of the imaginal stage. The rapid decrease early in the pupal stage was associated with an increase in degenerative cells (fig. 5).

The spheroidocytoids occurred in all stages, but never exceeded 12 percent of the hemocyte population. They seemed to be most prevalent in the second and third stadia, subsequently trending downward to disappear at about the prepupal stage. They fluctuated around 1 percent in the imaginal stage.

The orthospheroidocytes were found from the third stadium to about the time of pupation. They rose to more than 20 percent in the fifth stadium, and then decreased to 10 percent, increased again to about 30 percent at about the beginning of the prepupal stage, and then suddenly fell to near the zero level at about the time the prepupa began to taper. They continued near the 0.5-percent level to about the first day after pupation, and then were seen no more.

The metaspheroidocytes were present from about the beginning of the prepupal stage to about the middle of pupal life. They mounted suddenly and quickly above 40 percent at about the time of pupation, and then rapidly decreased, approaching zero at about the third day of pupal life.

The quick rise of the metaspheroidocytes was associated with the final rapid decrease of the orthospheroidocytes; and their subsequent rapid fall was associated with the marked increase of degenerating cells.

In the imaginal stage the paraspheroidocytes amounted to not more than 1 percent.

ERUPTIVE CELLS (CLASS IX)

The eruptive cells were present in all stages (fig. 5). They trended upward from the first stadium to fluctuate about the 30-percent level in the third stadium, and then trended downward until they approached zero at about the fourth day of pupal life, with a questionable increase at the seventh day of pupal life. During the imaginal stage the eruptive cells gradually rose to about 15 percent on the fifth day and then declined.

The rhegmatocytoids were present from the first stadium to the first part of the fifth, and were responsible for the form of the curve up to that time. After their initial rise they fluctuated about the 30-percent level in the third and fourth stadia, and then fell to near zero in the early part of the fifth stadium. Rhegmatocytoids appeared again in the imaginal stage, fluctuating at levels below 6 percent. They were also responsible for the questionable rise in percentage of eruptive cells on the seventh day of pupal life, when many seemed to be eosinophilic metaspheroidocytes.

The rhegmatocytes were found from about the beginning of the fifth stadium to the last part of the imaginal stage. They were responsible for the form of the curve for eruptive cells from the beginning of the fifth stadium to the fourth day of the pupal stage. Between the second and fifth stadia the rhegmatocytes occurred sporadically. During the imaginal stage they rose to about 10 percent on the fifth day and then decreased.

DEGENERATING CELLS (CLASS X)

Degenerating cells were found in all stages of development. Prior to the prepupal stage they fluctuated at values less than 2.5 percent, except in the third and fourth stadia, when they increased to not more than 7 percent. They also increased in the prepupal stage. By the fifth day of pupal life they had increased to more than 50 percent and still amounted to about 30 percent at the time of adult emergence. It is possible that they attained a maximum during the latter part of the pupal stage, for which differential counts were not made. They fluctuated around 8 percent during the first 5 days of imaginal life, but then increased.

PASSIVE-ACTIVE TRANSFORMATIONS AND THE FUSIFORM-NONFUSIFORM (F/N) RATIO

With the exception of the eruptive cells, the hemocytes in the smears were considered to have approximately the same form that they had in the circulating blood at the time the insects were heat-fixed. Most of them were in the passive form, but many showed indications of very slight transformation toward the active form. Such transformation appeared to be associated with larval molts, metamorphosis, and the presence of bacteria in the hemolymph. The indications of passive-active change included increased rounding (pls. I, A, d_2, H, de_{5-6}; II, A, gc; III, B, gc_{7-8}; IV, B, gc_{1-2}) or spreading (pl. II, A, df_4) of the hemocytes, increased raggedness of their nuclei, and irregularity of their cytoplasmic surface. These change were observed especially in the plasmatocytes and cystocytes. Cytoplasmic spreading also was exhibited by some of the vermiform cells, particularly from the prepupae and pupae. Occasionally some of the

cells appeared unusually smooth in outline (pl. IV, *F*, *d₁*) and exhibited differentiation of ectoplasm and endoplasm (pl. II, *A*, *df₄*; IV, *F*, *d₁*).

In a fusiform cell one manifestation of such passive-active transformation, observed particularly among the plasmatocytes and cystocytes, is the disappearance of its spindle ends and a rounding up or spreading of the cell. Both fusiform and nonfusiform cells occurred among the microcytes, proleucocytes, liocytes, liocytoids, plasmatocytes, and cystocytes. The ratio of the number of such cells that are fusiform to the number that are not fusiform, or the F/N ratio, should serve as an index to the occurrence of initial passive-active changes among them, the ratio exceeding unity if more than half were fusiform.

The changes in the F/N ratio in the different blood cells during the life of the insect are shown in figure 6. For the combined micro-
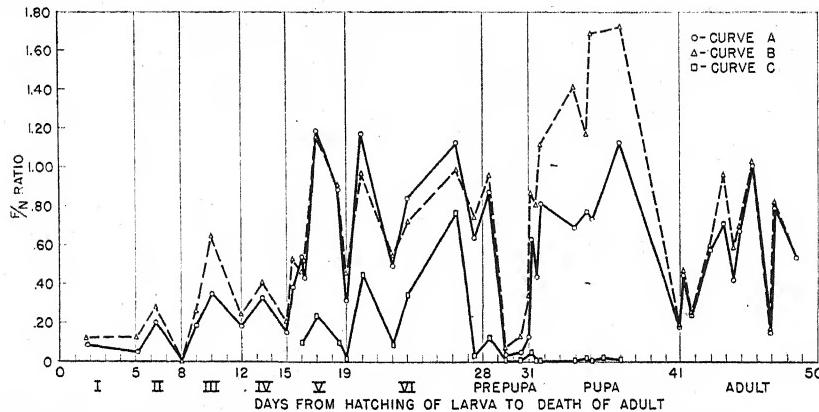

FIGURE 6.—Variation of fusiform-nonfusiform (F/N) ratio of hemocytes during development of *Prodenia eridania: A*, Microcytes, proleucocytes, liocytes, microplasmatocytes, mesoplasmatocytes, macroplasmatocytes, and cystocytes; *B*, microplasmatocytes, mesoplasmatocytes, and macroplasmatocytes; *C*, cystocytes.

cytes, proleucocytes, liocytes, microplasmatocytes, mesoplasmatocytes, macroplasmatocytes, and cystocytes (curve *A*) the ratio trended upward until in the fifth and sixth instars it fluctuated about the 0.80 value. In the prepupal stage it suddenly decreased to less than 0.05 but rose rapidly in the pupal period to about 0.80 and later to over 1.00. During the imaginal stage the ratio trended upward from about 0.20 to approximately 0.65, about which it fluctuated. The ratio tended to decrease at the larval molts, just prior to pupation, and at the beginning of the imaginal period. The decrease just prior to pupation was especially marked.

For the combined microplasmatocytes, mesoplasmatocytes, and macroplasmatocytes (curve *B*) the trend is similar, differing chiefly in the higher F/N values in the early instars and, especially, in the pupal period.

Only between the fourth stadium and the latter part of the pupal stage were sufficient cystocytes enumerated to give F/N values (curve *C*). The ratio trended upward to a maximum at the end of the sixth stadium and then decreased suddenly. After the middle of the prepupal period the value was close to zero.

The decrease in the F/N ratio at about the time of the molts, pupation, and emergence was particularly evident in the plasmatocytes throughout the life span and in the cystocytes from the fifth stadium to the prepupal stage. Microscopic observation indicated that at at the end of the sixth stadium the cystocytes showed passive-active transformation a little sooner than did the plasmatocytes. Whereas the plasmatocytes tended to regain their spindle form in the pupal stage, the cystocytes remained rounded. This largely accounts for the difference between curves *A* and *B* during the prepupal and pupal stages.

CHANGES IN THE HEMOCYTES ASSOCIATED WITH MOLTING

The hematocytes underwent slight, ill-defined changes in form at the time of the larval molts, particularly into the fourth, fifth, and sixth instars. The plasmatocytes, the cystocytes, and to a less extent other hemocytes, appeared slightly more rounded and spread than usual, their nuclei more irregular and ragged, and their chromatin masses more loosely knit. The cells seemed larger and less deeply stained, possibly because they were more thinly spread, although occasionally plasmatocytes appeared to be more rounded and more basophilic. Cytoplasmic vacuolization of the plasmatocytes also tended to be coarser and slightly more irregular. The surface of the cell, particularly of the plasmatocytes, seemed more irregular and sometimes exhibited ectoplasmic extension. Many of the orthospheroidocytes seemed larger, possibly because of spreading, and more like metaspheroidocytes.

CHANGES IN THE HEMOCYTES DURING METAMORPHOSIS

Most of the changes in the blood cells that have been described as occurring just before or during metamorphosis were associated with cell degeneration, development of new cells, and passive-active transformation.

The decline and disappearance of the spheroidocytes and cystocytes was associated with a rise in the percentage of degenerative cells. Since only cells so degenerate that they could not be definitely identified were classified as degenerating, the extent to which they represented spheroidocytes or cystocytes was not determined. Yet the various degrees of break-down observed among the spheroidocytes indicated that many of the degenerative cells had been spheroidocytes. Cellular break-down could not be so readily observed in the cystocytes, even when few cystocytes were left in the pupal blood smears.

The spheroidocytes in general showed the following changes during metamorphosis. Most of the orthospheroidocytes of the sixth instar transformed into the metaspheroidocytes of the prepupa by developing a greater cytoplasmic area relative to nucleus and a greater number of spheroids (pl. IV, *B*, hc_{5-7}). The spheroids became larger, and some of them slightly eosinophilic. Most of the metaspheroidocytes were conspicuous, roundish cells, bloated with spheroids. Frequently they contained very eccentric nuclei. Considerable agglutination occurred. Some cells contained, in addition to spheroids, a deeply basophilic, plastidlike, cytoplasmic inclusion. Occasionally a similar basophilic body adhered to the surface of a metaspheroidocyte.

At about the middle of the prepupal stage some of the metaspheroidocytes began to degenerate. In some cells the signs of degeneration

included a decrease in intensity of staining reaction (achromophilia), greater spreading of the cell in the smear, loss of cytoplasmic structure, and nuclear raggedness. In many of these cells the nucleus tended to break up into irregular chromosomelike masses (karyorrhexis). Frequently the degenerating cytoplasm contained areas that were slightly eosinophilic. These degenerating cells usually contained few recognizable cytoplasmic inclusions other than the spheroids and possibly a basophilic body. An almost completely degenerated cell consisted of an area of spread, nearly structureless, achromophilic cytoplasm and a degenerated or degenerating nucleus.

Other degenerating metaspheroidocytes contained various numbers of cytoplasmic inclusions, ranging from granular to larger than nuclear size. The granules were usually eosinophilic, but in the larger inclusions the staining reaction ranged from deeply basophilic to deeply eosinophilic. Whole cells frequently showed a slight to intense eosinophilic reaction. Many of the intensely eosinophilic metaspheroidocytes resembled rhegmatocytes but were larger (pl. IV, B, hc_{11-13}). A completely degenerated cell, well loaded with inclusions, appeared as a roundish mass of variably stained, eosinophilic debris, the components of which could hardly be distinguished. Many of the inclusions in these metaspheroidocytes seemed to have been phagocytized material; for example, a cell occasionally contained what was apparently a fragment of cytoplasm from a cystocyte, perhaps a plastid. Some of the basophilic inclusions resembled bodies that were found free in the plasma, especially when it contained liberated adipose cells.

In many of the metaspheroidocytes, particularly after they had begun to show degenerative changes, the spheroids became as large as the cysts in the cystocytes or larger. In some of the degenerating metaspheroidocytes the nuclei tended to become more compact and more deeply staining (pycnosis) rather than spread, achromophilic, and fragmented. In some cells the spheroids had an altered texture and became less distinct individually. Four degenerating metaspheroidocytes are shown in plate IV, B, hc_{1-4}.

Although the cystocytes showed less marked cytological changes during metamorphosis than did the spheroidocytes, many of them appeared to have become rounded (pls. II, A, gc; III, B, gc_{7-8}; IV, B, gc_{1-2}), as was shown by actual counts (fig. 6) to have been the case. The nuclei of some cystocytes became very eccentric, and some cells appeared unusually small (pl. III, B, gc_7). At about the beginning of prepupal tapering cystocytes began to agglutinate with each other or with other hemocytes, especially spheroidocytes and deeply basophilic cells (probably plasmatocytes). More marked agglutination occurred during pupal development. In the late sixth stadium and the prepupal stage the intercystic basophilic cytoplasm became more conspicuous. In the very early pupa it ceased to appear as a kind of basophilic network separating the eosinophilic inclusions and assumed the aspect of irregular, stringy material, frequently appearing as loops (pls. II, A, gc; III, B, gc_{7-8}) lying in an eosinophilic field in which the individual inclusions were inconspicuous. Cell debris, apparently originating from adipose cells, occurred in the plasma and frequently was associated with the cystocytes.

As has been stated, many of the plasmatocytes, like the cystocytes, tended to round up during the prepupal stage but, unlike the cysto-

cytes, they tended to resume their spindle shape in the pupa. Many of the pupal plasmatocytes, however, both rounded and fusiform, showed ectoplasmic irregularities. Occasionally, plasmatocytes contained inclusions, usually basophilic, that probably had been phagocytized. Degeneration of plasmatocytes seemed to involve a loss of cytoplasmic structure, a decreased staining reaction, and raggedness or fragmentation of nucleus, although occasionally plasmatocytes that stained brilliantly were present during metamorphosis. The plasmatocytes became more grossly vacuolized (pl. I, A, d_2; H, de_{5-6}) and, in general, began to exhibit the appearance characteristic of the imaginal plasmatocytes, particularly in the latter part of the pupal stage.

Prior to and early in metamorphosis the spheroidocytes and sometimes other cells frequently contained colorless but brilliant and highly refractive material. Sometimes the refractive material consisted of crystals (pl. IV, A, hb_1), but at other times it seemed to be present as colorless droplets.

In addition to the recognizable hemocytes, the plasma contained large cell-like masses (pl. IV, G, m), interpreted as liberated, circulating fat-body cells, consisting of many plastidlike bodies and larger vacuoles about a nucleus (n). Liberation of fixed adipose cells during metamorphosis has been reported by others, including some of the earlier investigators (27). The plastidlike bodies varied in staining reaction from basophilic to eosinophilic and some, free in the plasma, resembled the basophilic inclusions in the metaspheroidocytes. Some that were eosinophilic slightly resembled the cysts, in the cystocytes, although they tended to be larger. In addition to the free adipose cells, and the plastidlike bodies originating from them, body fluid contained true blood plastids, derived from hemocytes. Plastids, recognizable as bits of cytoplasm from spheroidocytes, cystocytes (pl. III, B, k), and plasmatocytes (pl. IV, F, k), did occur in the plasma, not only during metamorphosis, but also in other stages of the life cycle. In plate IV, F, d_2, is a plasmatocyte in the process of plastid formation.

Degeneration of podocytes, vermiform cells, and oenocytoids seemed to involve chiefly a gradual loss of cell structure and staining reaction. Some of the vermiform cells showed surface irregularities and seemed to become more spread or sheetlike, probably an indication of passive-active transformation. The nuclei of the oenocytoids frequently seemed to fragment or to be partly extruded from the cell.

Many of the pupal hemocytes, especially those that seemed degenerative, tended to stain muddily and opaquely rather than brilliantly and translucently, as did the larval hemocytes. Whether the decreased brilliancy of staining was caused entirely by the changes in the cells or in part by changes in the plasma was not determined.

IDENTIFICATION OF STAGE OF DEVELOPMENT BY MEANS OF HEMOLYMPH SMEARS

To indicate the extent to which the hemolymph picture could be used to identify the stages of development of *Prodenia eridania*, the author determined as closely as possible the stages of 100 insects from an examination of their hemolymph smears. The smears were placed on and removed from the microscope stage by a coworker (Sam C. Munson), who also brought the hemocytes into initial focus. Since the slides used in the test were some of those made in the course of this

study, precautions were taken to prevent any recognition of the smears other than that gained from the microscopic observation of the hemocytes.

In this test 76 percent of the smears were identified correctly as to stage of development. Of the identifications 82 percent were correct to within 24 hours and 88 percent to within 48 hours of the true stage. Twelve percent were correct to within 6 hours of the true age within a given stage, 24 percent to within 12 hours, 53 percent to within 24 hours, and 66 percent to within 48 hours.

RELATIONSHIPS BETWEEN DIFFERENT KINDS OF HEMOCYTES

The morphological and developmental relationships between the different kinds of hemocytes can be estimated on the basis of the results that have been described. Evidence of these relationships is supplied by cytological comparisons, the time in the life cycle at which different cell types appear, change, or disappear, the occurrence of mitotic figures, and passive-active transformation. The relationships indicated in figures 7–10 are considered only first approximations.
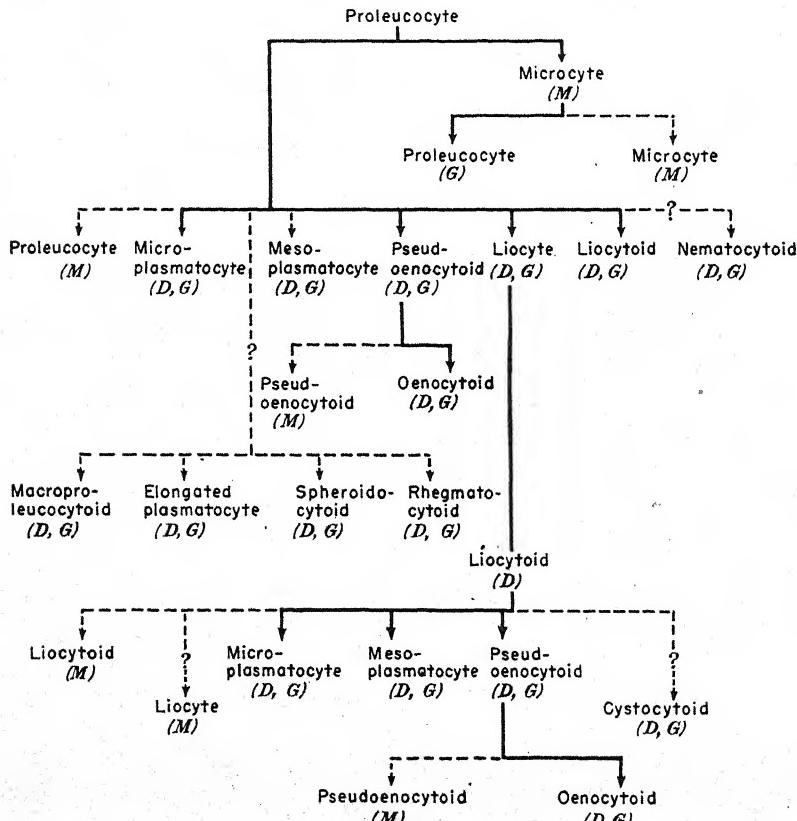
FIGURE 7.—Scheme showing relationship between proleucocytes, microcytes, plasmatocytes, oenocytelike cells, and some other hemocytes. The unbroken lines show the main trends of development; the broken lines, other relationships. See text for further explanation.

The relationships between the different kinds of hemocytes indicated in the schemes shown may be considered as slightly overlapping sections of a single larger plan. In each scheme certain main trends of development are shown by heavy lines; other relationships are represented by light lines. The direction of development is shown by the arrows. The kinds of developmental change are indicated by the letters in parentheses, D referring to differentiation, G to growth, M to mitosis, and T to passive-active transformation. Mitosis is indicated for only those types of hemocytes in which mitotic figures were actually observed. For example, M under microcytes indicates that these cells can be produced from proleucocytes or from microcytes by mitotic division, and G under proleucocytes indicates that microcytes can change into proleucocytes by growth processes. The question marks indicate possible but less probable relationships.

The first scheme (fig. 7) shows the main trends of development that extend, either directly or indirectly, from the proleucocytes to the microcytes, certain plasmatocytes, oenocytelike cells, and chromophiles.
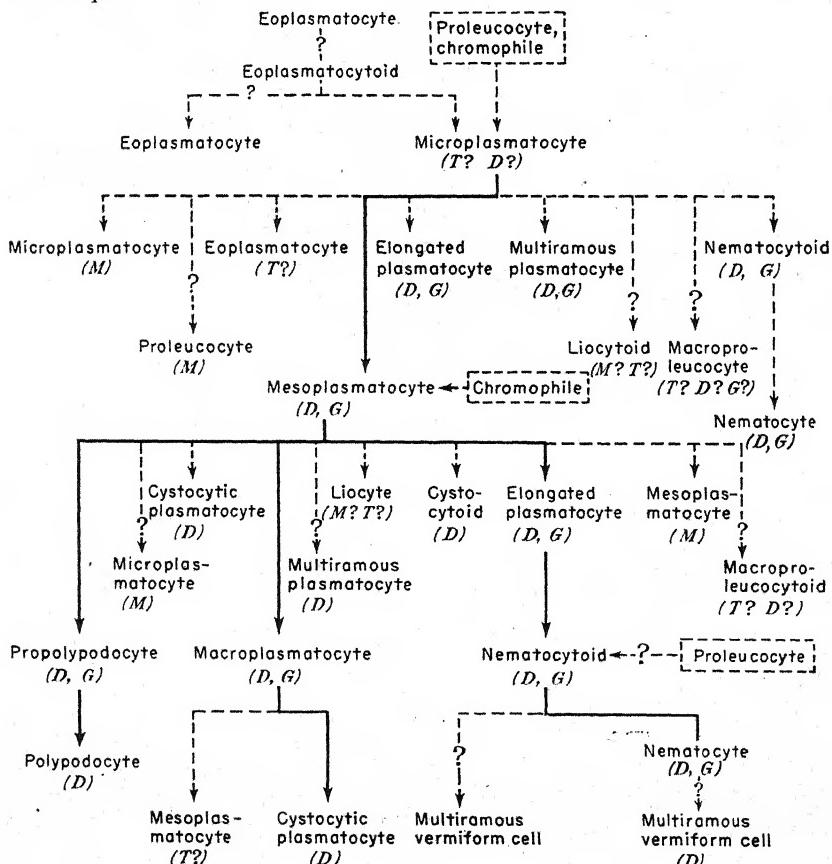
FIGURE 8.—Scheme showing relationship between plasmatocytes, podocytes, vermiform cells, and some other hemocytes. The unbroken lines show the main trends of development; the broken lines, other relationships. See text for further explanation.
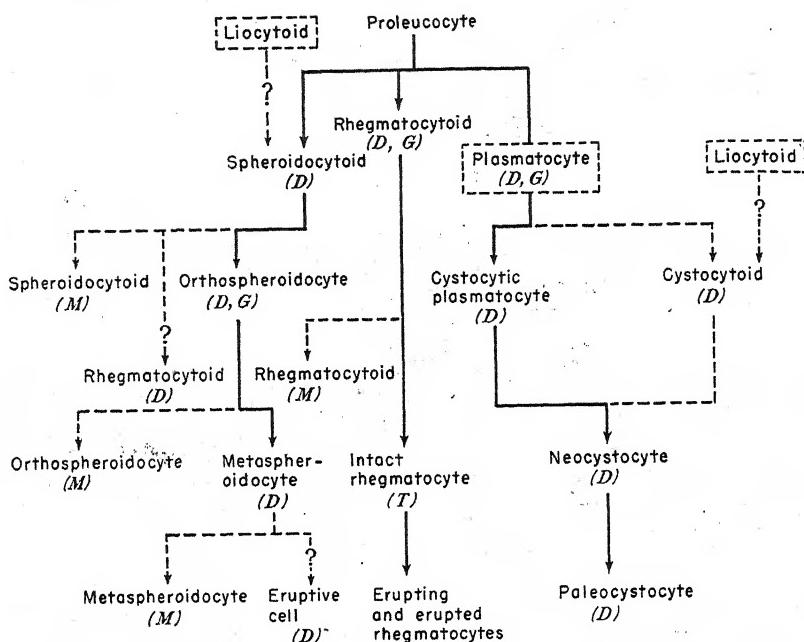
FIGURE 9.—Scheme showing relationship between proleucocytes, cystocytes, spheroidocytes, eruptive cells, and some other hemocytes. The unbroken lines show the main trends of development; the broken lines, other relationships. See text for further explanation.
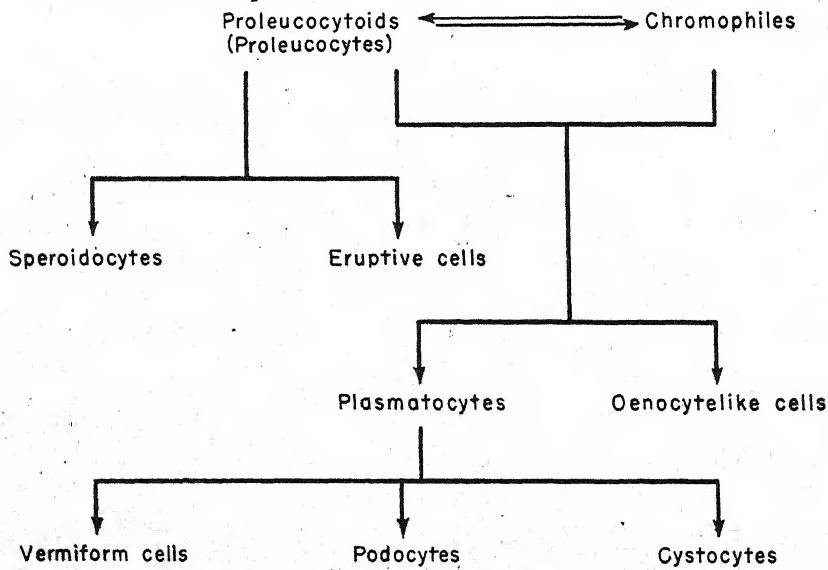
FIGURE 10.—Scheme showing relationship between the classes of hemocytes. See text for further explanation.

The main relationships of the plasmatocytes, podocytes, and vermiform cells and a number of associated relationships are indicated in figure 8.

The main lines of development of the cystocytes, spheroidocytes, and eruptive cells are represented by the scheme shown as figure 9.

The scheme shown as figure 10 represents the main relationships of the classes of hemocytes. The proleucocytes, rather than the microcytes, are related by transitional or developmental forms to the cells of the other classes. Both plasmatocytes and oenocytelike cells may be derived from either proleucocytoids or chromophiles.

DISCUSSION

PHASES OF DEVELOPMENT OF THE INSECT

The results of this study show that the development of *Prodenia eridania* can be divided according to its blood picture into early larval, late larval, metamorphic, and imaginal phases. The early larval phase, extending from the time of hatching to about the beginning of the fourth or fifth stadium, is characterized by relatively large percentages of proleucocytes, liocytes, liocytoids, rhegmatocytoids, eoplasmatocytes, eoplasmatocytoids, microplasmatocytes, spheroidocytoids, and pseudoenocytoids. In other words, this is a developmental or transitional phase, characterized by the predominance of hemocytes that can be considered primitive, transitional, and perhaps embryonic forms. Occasionally, however, third instars were found to possess plasmatocytes, cystocytes, and vermiform cells, which gave their hemolymph smears a partial fifth- or sixth-instar aspect.

The late larval phase, extending from about the beginning of the fourth or fifth stadium to within the prepupal period, is characterized by predominance of the cystocytes, orthospheroidocytes, larval mesoplasmatocytes and macroplasmatocytes, and larval rhegmatocytes. These may be considered to be the special larval hemocytes, types that during larval and prepupal life trend upward to a maximum and disappear during metamorphosis.

The metamorphic phase is a transitional phase, extending from the end of the sixth stadium to the emergence of the imago. It is characterized by the rise to a maximum and the subsequent decrease and disappearance of the metaspheroidocytes and eosinophilic cystocytes, the marked rise of degenerative cells, and the beginning of the development of imaginal hemocytes. The oenocytelike cells, podocytes, vermiform cells, and macroplasmatocytes disappear, and the microplasmatocytes become more numerous than the mesoplasmatocytes. The indications are that the larval plasmatocytes disappear and the imaginal plasmatocytes become newly developed. In the latter part of this phase the number of hemocytes becomes very low.

The imaginal phase is characterized by the great predominance of the imaginal plasmatocytes.

The larval blood picture reaches full development in the late larval phase, but is destroyed during the transitional metamorphic phase and is replaced by the newly developing imaginal blood picture. This is in accordance with a previous report (26) that the total cell count is higher in the larvae than in the imagoes of some holometabolous insects, and with the suggestion that, in contrast to heterometabolous insects, holometabolous insects develop different larval and imaginal bloods. The present results indicate further that certain types of hemocytes, such as the plasmatocytes of *Prodenia eridania*, may be common to both larval and imaginal periods, although a

given type may have a slightly different morphological appearance in the larva and imago.

COMPARISON OF THIS AND OTHER CLASSIFICATIONS

The normal hemolymph picture of *Prodenia eridania* is more complex than similar pictures of a number of other Lepidoptera examined at various times. From one viewpoint, the blood picture of an insect is more complex than that of the average mammal. The circulating blood of the normal mammal carries almost entirely mature leucocytes and erythrocytes. The developmental stages of these cells, that is, the various types of leucoblasts and erythroblasts, are confined almost entirely to the leucopoietic and erythropoietic tissues. The circulating mammalian blood cells do not normally undergo mitotic division. On the other hand, since the circulating hemolymph of *Prodenia eridania*, and probably of most other insects, contains not only mature hemocytes but also transitional forms, mitotic figures, and perhaps primitive forms, it is analogous to the blood plus the leucopoietic tissues of the mammal.

The name "proleucocyte" is not considered appropriate, because "leucocyte" is generally used with reference to vertebrate white blood cells and "hemocyte" appears to have an established usage relative to the cells in insect hemolymph (*29, p. 233*). It has been included tentatively in this classification because it is well established and refers to a generally recognized type of hemocyte. "Prohemocyte" eventually may be preferred.

It is frequently difficult to classify a borderline or transitional cell. In some instances the decision is almost arbitrary, but the percentage of such cells that are doubtfully classified is not great. Table 3 indicates that a differential count can be repeated with considerable success, insofar as recognition of type is concerned. Although no attempt has been made in this study to obtain a quantitative estimate of counting error, it is believed that differential counts of *Prodenia eridania* hemocytes involve errors comparable to those found in connection with counts of vertebrate white blood cells.

Available knowledge of types of hemocytes does not permit a very close comparison between hemocyte classifications that pertain to different orders of insects. This classification, therefore, will be compared mainly with other classifications that apply to Lepidoptera.

Most of the classifications of insect hemocytes developed from studies of hemolymph smears, including those by Busnel (*3, p. 125*), Cuénot (*4*), Muttkowski (*18*), Hollande (*6, 7*), Paillot (*20*), Pilat (*23*), and Bogojavlensky (*2*), have been based on smears prepared without deliberate use of anticoagulant procedures. Hufnagel (*9*) and Jackson (*11*),[8] however, used preliminary heat fixation. Mathur and Soni (*14*) used acetic acid vapor as a preliminary fixative. Inadvertent anticoagulation effects probably occurred, at times, in the techniques used by Hollande and Bogojavlensky. Rooseboom (*25*) recognized the desirability of hemolymph fixation prior to sampling, but apparently did not deliberately use an anticoagulant procedure in the preparation of smears.

It has long been known that the hemocytes of insects and other invertebrates play a role in hemolymph coagulation that involves changes both in their form and the degree of their agglutination,

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[8] The original thesis was seen by the author of the present paper.

Newport (*19*) in 1845 observed that the "oat-shaped corpuscles" underwent certain rapid changes in form, particularly in hemolymph removed from the insect's body, and pointed to this as one cause of the diversity in the descriptions of the form of the corpuscle given by various observers. The failure to take into account the form changes of hemocytes is still one cause of confusion in insect hematology. Therefore, it is not easy to make a close comparison between this classification and other classifications of lepidopterous hemocytes.

Partial or complete passive-active transformations of hemocytes can take place under certain physiological conditions not involving loss of hemolymph and under certain conditions of infection (*22*) and poisoning (*35*). If conditions favoring transformation and agglutination should prevail in an insect at the time of sampling and smearing, anti-coagulant heat fixation should preserve not only the passive but also the active form. The information now available indicates that, in the normal insect, most of the circulating hemocytes are in their passive form or very nearly so. In comparing this classification with pre-existing classifications, it should be borne in mind that usually a comparison is being made between cells mostly in their passive form with cells that may have undergone extensive transformation.

A number of investigators have utilized the classifications of Hollande (*7*) and Paillot (*20*), either with or without modifications. Paillot's classification is based on relative nuclear-cytoplasmic dimensions, the presence or absence of cytoplasmic spherules, and the recognition of the oenocytoid of Poyarkoff (*24*) and Hollande (*7*) as a distinct type. This classification is far too simplified to be of much help in identifying the hemocytes of *Prodenia eridania*, whether they are in their passive or active form. It offers no means of distinguishing between most of the types and classes of cells that can be identified by the present classification. Most of the proleucocytoids, smooth-contour chromophiles, plasmatocytes, and podocytes in their passive state would be macronucleocytes according to Paillot's classification. The other cells of these types, the spheroidocytes, the rhegmatocytes, and at least some of the cystocytes would be included in Paillot's micronucleocytes. A single cell, for example, a plasmatocyte, might be a macronucleocyte when in its passive form and a micronucleocyte when in its active form and widely spread in a smear. Paillot's macro- or micronucleoytes with spherules would have to include the cystocytes, the spheroidocytes, many of the rhegmatocytes, and probably any of the proleucocytoids, smooth-contour cells, plasmatocytes, and podocytes that happened to contain glycogen vacuoles. The present classification utilizes nuclear-cytoplasmic dimension in identifying some cell types—for example, the micro-, meso-, and macroplasmatocytes—in conjunction with other characters, but this factor is not sufficiently distinguishing to be used alone. None of Paillot's types except the oenocytoids, which he (*21*) had previously called oenocytes, are analogous to the types herein described.

Hollande's (*7*) proleucocytes and oenocytoids, the latter of which he earlier called lymphocytes (*6*), are analogous to the types of the same names in the present classification. His phagocytes, which he says are frequently fusiform, probably include plasmatocytes and, if all phagocytic cells are to be classified as this type, might have to include liocytoids, spheroidocytes, and perhaps microcytes, according to observations of phagocytic activity made during the present study.

But what Hollande called phagocytes probably correspond to the fusiform plasmatocytes that happen to retain their elongated, passive form in an air-dried smear of living hemolymph prepared from an insect without the use of an anticoagulant procedure.   His description of granular leucocytes has not been found to fit any cell type in the present classification, although it might be applicable in part to the spheroidocytes. His description of lepidopterous "cellules à sphérules" seems to indicate the rhegmatocytes, particularly in that the contents of the spherules can be liberated into the plasma.   None of Hollande's other types appear to be identical with types in the present classification.

Bogojavlensky (*2*) described the hemocytes of Orthoptera and Lepidoptera. His descriptions of lepidopterous hemocytes were based upon a study of *Bombyx mori* (L.), and to a less extent of *Vanessa urticeae* (L.), *Dicranura vinula* (L.), *Phalera bucephala* (L.), *Malacosoma neustria* (L.), *Macrothylacia rubi* (L.), *Mamestra pisi* (L.), *Cossus cossus* (L.), *Sphinx ligustri* L., and *Galleria mellonella* (L.). He examined the blood in vitro, in hanging-drop preparations, in stained smears, and in histological sections. He did not deliberately employ anticoagulant procedures. He observed changes in the forms of the hemocytes at the time of hemolymph sampling, but did not base his classification upon a separation of passive and active forms. He distinguished five types of hemocytes—hemocytoblasts, spindle-shaped leucocytes, micronucleocytes, spherocytes, and oenocytoids. The hemocytoblasts correspond to Hollande's proleucocytes, the spindle-shaped leucocytes to the phagocytes of Hollande and the macronucleocytes of Paillot, the micronucleocytes to Paillot's micronucleocytes, the spherocytes to Hollande's "cellules à sphérules," and the oenocytoids to the oenocytoids of Poyarkoff and Hollande. Bogojavlensky's hemocytoblasts and spherocytes seem to be analogous, respectively, to the proleucocytes and eruptive cells of the present classification. His spindle-shaped leucocytes would correspond to those proleucocytoids, smooth-contour chromophiles, and plasmatocytes that would retain their passive fusiform shape in hematological preparations such as he used, as well as to the vermiform cells and some of the podocytes and cystocytes. His micronucleocytes would seem to correspond to the spheroidocytes. Bogojavlensky's classification has no place for some of the types of hemocytes herein described, but his descriptions can be interpreted in terms of the present classification more easily than can those of Hollande, Paillot, and a number of other investigators. This may be partly because Bogojavlensky used moist smears and made a special effort to attain rapid fixation prior to staining.

Hufnagel (*9*) described five types of hemocytes in *Hyponomeuta padella* (L.)—proleucocytes, young leucocytes, old or phagocytic leucocytes, leucocytes with fatty inclusions, and granular leucocytes. The young leucocytes probably would include the plasmatocytes, liocytoids, and perhaps the liocytes and podocytes of the present classification. Parts of the descriptions of the old or phagocytic leucocytes and the leucocytes with fatty inclusions imply that the former and possibly the latter correspond in part to the spheroidocytes. But Hufnagel's classification does not permit identification of many of the hemocyte types of *Prodenia eridania*.

Two of the types of lepidopterous hemocytes recognized by Cuénot (4) apparently correspond to oenocytoids and rhegmatocytes. Cuénot's other types are obviously depicted on the basis of hemocytes in an active state and are not easily compared with the types herein described. The three kinds of hemocytes of *Ephestia kuehniella* Zell. described by Blaustein (1) would probably correspond to proleucocytes, plasmatocytes, and spheroidocytes. The classifications used by Kollmann (13), Metalnikov (16), Iwasaki (10), and a number of others are not applicable to *Prodenia eridania* and are not very comparable to the present classification, except that, in general, forms corresponding to proleucocytes and oenocytoids and cells containing vacuoles, or spherules, are recognized. Muttkowski (18), however, observed certain very chromophilic cells that might include the present liocytes and more deeply basophilic liocytoids. Some of the spheroidal cells described by Metalnikov and Chorine (17) seem to correspond to rhegmatocytes.

ORIGIN, FATE, AND POSSIBLE FUNCTIONS OF THE HEMOCYTES

The presence of several kinds of hemocytes in recently hatched *Prodenia eridania* indicates that they originated and were differentiated during embryonic development. The presence of mitotic figures in the various stages of the insect's development shows that multiplication of hemocytes occurs in the circulating hemolymph. This is in accordance with the observations of most investigators. The mitotic index did not become very high even when the hemolymph contained the largest percentages of cells interpretable as young and transitional forms (fig. 2, *A*). This might indicate either that some of the cells are formed by division of noncirculating cells or that too few hemocytes were enumerated. It is of interest, nevertheless, that a greater number of mitotic figures were not observed in the hemolymph during the transitional (early larval and metamorphic) phases of development. Whether the hemocytes of *Prodenia eridania* have postembryonic origin exclusively in the hemolymph has not been shown in the present study.

These results indicate that the fate of the hemocytes in the circulating blood of *Prodenia eridania* includes degeneration, development into a more mature form, change into a different type, phagocytosis by other hemocytes, and perhaps loss to the tissues. Normally a slight amount of cell degeneration may occur at various times during the life cycle of the insect, although most takes place in the pupal stage (fig. 5). It is not known to what extent the absolute number of hemocytes results from a balance between degeneration and mitotic reproduction. Evidence for the phagocytosis of hemocytes by other hemocytes was slight and was obtained only during the metamorphic phase.

Insect hemocytes have phagocytic ability (22), participate in hemolymph coagulation (32), and under certain conditions agglutinate in vivo, forming capsules about foreign material (8, 5). The present as well as previous but unpublished observations indicate that a variable but normally small degree of agglutination may occur in the circulating blood of a normal insect. Hemocyte participation in wound healing has also been demonstrated (28, 5). Passive-active transformation is involved in these activities, all of which may be evoked in the defense of the organism.

Insect hemocytes can also cast off nonnucleated portions of their cytoplasm, referred to herein as plastids. In the present study plastids were observed during all stages of development of the southern armyworm. An increase of plastid formation in the blood of this insect may follow the administration of certain insecticides (*35*). The significance of plastid formation by hemocytes is not yet known, but no doubt it is the manifestation of a definite cellular activity. It might indicate a purgative or secretory action, as might the eruptive activity of the rhegmatocytes.

The glycogen-storage function of many of the hemocytes of *Prodenia eridania* has already been reported (*34*).

It has been shown that certain types of hemocytes characteristic of the larva—for example, the oenocytoids and cystocytes—increase to a maximum at about the beginning of pupation and then decline and disappear in the pupal stage. Other cells are present in all stages of the life span, such as the plasmatocytes, although the larval plasmatocytes do disappear in the pupal stage and the imaginal plasmatocytes apparently are developed anew. The larval cells that are irreplaceably lost during metamorphosis might perform one or more functions especially characteristic of larval life or metamorphosis, whereas other cells, such as the plasmatocytes, perform general functions required by the insect through its life. It is thus possible that the hemocytes fall into two categories, those that perform special and those that perform general functions.

SUMMARY AND CONCLUSIONS

The blood cells, or hemocytes, present in all stages of the life cycle of the southern armyworm (*Prodenia eridania* (Cram.)) have been studied, classified, and differentially counted. They fall into 10 classes, as follows: Proleucocytoids, smooth-contour chromophilic cells, oenocytelike cells, plasmatocytes, podocytes, vermiform cells, cystocytes, spheroidocytes, eruptive cells, and degenerating cells. The 10 classes are subdivided into the following 32 types: Microcytes, proleucocytes, liocytes, liocytoids, pseudoenocytoids, oenocytoids macroproleucocytoids, eoplasmatocytes, eoplasmatocytoids, microplasmatocytes, mesoplasmatocytes, macroplasmatocytes, elongated plasmatocytes, multiramous plasmatocytes, propolypodocytes, polypodocytes, nematocytoids, nematocytes, multiramous vermiform cells, cystocytoids, neocystocytes, paleocystocytes, cystocytic plasmatocytes, spheroidocytoids, orthospheroidocytes, metaspheroidocytes, paraspheroidocytes, rhegmatocytoids, rhegmatocytes, achromophiles, hyaline cells, and degenerating cells.

The types of hemocytes are described and their variants indicated. Mitotic division in a number of these types was noted in all the stages of the insect's development. Some of the blood cells exhibited slight alterations of a passive-active character at about the time of the molts and showed more marked transformations during metamorphosis.

The changes of the different kinds of hemocytes served as a basis for dividing the life cycle into early larval, late larval, metamorphic, and imaginal phases. The early larval phase is characterized by the predominance of transitional, primitive, and perhaps embryonic types of blood cells. The late larval phase is characterized by the marked development of special larval hemocytes that attain a maximum in numbers and development at about the beginning of pupation and

disappear in the pupal stage. The metamorphic phase, including prepupal and pupal stages, is a transitional phase, marked by the presence of metaspheroidocytes, the attainment of a maximum in percentage of some of the types, the eventual disappearance of virtually all the larval hemocytes, and apparently the initial appearance of the imaginal hemocytes. The imaginal phase is characterized by the development of the imaginal hemocytes, most of which are plasmatocytes.

The ratio of transformable cells that are fusiform to those that are nonfusiform, or the F/N ratio, was used to indicate the tendency of these cells to undergo passive-active rounding. Rounding took place particularly at about the time of the molts, pupation, and emergence. Prepupal rounding of the plasmatocytes but not of the cystocytes appeared to be reversed later.

The interrelationships of the different types and classes of hemocytes are discussed and schematically presented. It is indicated that the chromophiles, spheroidocytes, eruptive cells, plasmatocytes, and oenocytelike cells are derivable from the proleucocytes, some of which are derivable from the chromophiles, and that the vermiform cells, podocytes, and cystocytes can be derived from the plasmatocytes. The comparison of this with other classifications applicable to the hemocytes of Lepidoptera is discussed, as are the origin, fate, and possible functions of the hemocytes of *Prodenia eridania*.

In brief, the definitely known functions of the various hemocytes of *Prodenia eridania* may be summarized as (1) phagocytosis, (2) participation in hemolymph coagulation, (3) agglutination in vivo, including the formation of capsules, (4) plastid formation, (5) eruption or sudden liberation of material into the plasma under certain conditions, and (6) glycogen storage. Unpublished observations indicate also that under certain conditions they may contain deposits of fat. Because of their ability to undergo passive-active transformation they probably also participate in wound healing. The present results further suggest that during the life of the insect some hemocytes perform special and others general functions.

It is possible with a success of about 75 percent of trials to identify the stages of development of *Prodenia eridania* merely by inspection of its hemocytes in hemolymph smears.

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EXPLANATORY LEGEND FOR PLATE I

A, aa_1, Fusiform microcytes, and d_1, plasmatocyte, from male pupa 22 hours old; aa_2, microcyte (probably newly formed), and d_2, plasmatocyte, from female pupa not over 1 day old. Light area by nucleus of d_1 is probably a glycogen inclusion.

B, ab_{1-2}, Proleucocytes from a first instar about 44 hours old; ab_{3-4}, proleucocytes (probably transitional), *dd,* microplasmatocyte, all from a fifth instar.

C, ba_{1-4}, Liocytes, *bb,* liocytoid, *d,* plasmatocyte, *df,* macroplasmatocyte, *eb,* polypodocyte, and *h,* spheroidocyte, all from a sixth instar 100 hours old. One or more of the large light areas in *df* and the one near the nucleus in *eb* probably are glycogen inclusions.

D, ba_{1-2}, Liocytes, bb_{1-4}, liocytoids, all from a sixth instar 100 hours old.

E, Oenocytoids: cb_{1-3}, cb_7, all from young fifth instars; cb_4, cb_8, cb_9, and cb_{11}, all from a prepupa beginning to taper; cb_{5-6}, both from a young sixth instar; cb_{10}, from a second instar 25–39 hours old. Peculiar cytoplasmic texture in cb_{3-4} and eosinophilic matter in cytoplasm of cb_7 and cb_{11} shown only faintly.

F, db_1, Eoplasmatocyte from a third instar; db_2, eoplasmatocyte, and *ia,* rhegmato-cytoid, from a second instar 25–39 hours old.

G, Microplasmatocytes: dd_{1-2}, from a third instar; dd_3 and dd_{12}, from a fifth instar; dd_{4-5} and dd_{13}, from a second instar 25–39 hours old; dd_{6-7}, from female adult $4\frac{1}{2}$–21 hours old; dd_8, from a first instar about 44 hours old; dd_9, from a female adult $97\frac{1}{2}$–114 hours old; dd_{10}, from a young sixth instar; dd_{11}, from a young fifth instar.

H, dd, Microplasmatocyte, de_1, mesoplasmatocyte, and df, macroplasmatocyte, all from a female adult $97\frac{1}{2}$–114 hours old. The other mesoplasmatocytes are de_2 and de_4, from female adult $4\frac{1}{2}$–21 hours old; de_3, from a young sixth instar; de_{5-6}, from a prepupa beginning to taper; de_{7-9}, from a first instar about 44 hours old; de_{10}, from a male pupa $21\frac{3}{4}$ hours old.

Explanatory Legend for Plate II

A, dd_1, Microplasmatocyte, de_1, mesoplasmatocyte, and df_{1-3}, macroplasmatocytes, all from a pupa $21\frac{3}{4}$ hours old; de_2, mesoplasmatocyte, df_{4-5}, macroplasmatocytes, and gc, paleocystocyte, all from a prepupa beginning to taper; df_{6-8} and df_{11-12}, macroplasmatocytes, and hb_{1-2}, orthospheroidocytes, all from sixth instars 100 hours old; dd_2, microplasmatocyte, df_{9-10}, macroplasmatocytes, and ib_1, rhegmatocyte, all from female adults $4\frac{1}{2}$–21 hours old; df_{13-14}, macroplasmatocytes, and ib_2, rhegmatocyte, all from a female adult $70\frac{1}{4}$ hours old. The largest light areas in de_1, df_{1-3}, and df_{11-12} are probably glycogen inclusions. The erupted cytoplasm of ib_1 is only faintly visible.

B, dg, Elongated plasmatocyte from a young sixth instar. The extremities of the spindle ends are not visible.

C, dd, Microplasmatocyte, hb, orthospheroidocyte, and dh, multiramous plasmatocyte, all from a sixth instar 100 hours old.

D, ea, Propolypodocyte, and eb, polypodocyte, from a female pupa 24–48 hours old. A considerable length of the cytoplasmic extensions is not visible.

Explanatory Legend for Plate III

A, fa_{1-2}, Nematocytoids, de, mesoplasmatocyte, df_{1-2}, macroplasmatocytes, all from a male pupa $21\frac{3}{4}$ hours old; fb, nematocyte, bb, liocyte, and eb, polypodocyte, all from a prepupa; gc, paleocystocyte, de, mesoplasmatocyte, and hb, four orthospheroidocytes, all from a sixth instar 100 hours old. The large light areas next to the nuclei in fa_{1-2} and in de are probably glycogen inclusions.

B, gc_{1-3}, Paleocystocytes, dd_1, microplasmatocyte, hb, orthospheroidocyte, gc_{4-6}, paleocystocytes, gd, cystocytic plasmatocyte, d_{1-3}, plasmatocytes, dd_2, microplasmatocyte, and ib, rhegmatocyte, all from a greatly tapered prepupa; gc_{7-8}, paleocystocytes from a male pupa $21\frac{3}{4}$ hours old; gb, neocystocyte, gc_9, paleocystocyte, and k, plastid, all from a prepupa beginning to taper. The plastid k is merely lying against the paleocystocyte gc_9 and is not a part of it.

C, ha_{1-4}, Spheroidocytoids, d, plasmatocyte, and ia, rhegmatocytoid, all from a second instar 25–39 hours old; ha_{5-6}, spheroidocytoids from a third instar; ha_7, spheroidocytoid from a first instar about 44 hours old; ha_8, spheroidocytoid from a young fifth instar. The spheroids or vacuoles in ha_{1-8} appear as small, very faint, light areas in the cytoplasm.

Explanatory Legend for Plate IV

A, hb_1, Group of orthospheroidocytes; hb_{2-5}, orthospheroidocytes, and dd, microplasmatocyte, all from a young fifth instar; hb_{6-9}, orthospheroidocytes from a prepupa beginning to taper. The dark part of hb_2 is the nucleus; about this the light vacuolated cytoplasm shows very faintly.

B, hc_{1-4}, Metaspheroidocytes, de_{1-2}, mesoplasmatocytes, df, macroplasmatocyte, gc_{1-2}, paleocystocytes, ib_1, rhegmatocytes, all from pupa $21\frac{3}{4}$ hours old; hc_{5-7}, metaspheroidocytes, from prepupa beginning to taper; hc_{8-13}, metaspheroidocytes, and ib_2, rhegmatocyte, from a female pupa 1 day old. A lump of material, probaly phagocytized, is in a vacuole in cell hc_5.

C, hd, Paraspheroidocyte from female adult $97\frac{1}{2}$–114 hours old.

D, ia_{1-3}, Rhegmatocytoids, dd, microplasmatocyte, all from a third instar. The erupting matter about cell ia_3 is red.

E, ib_{1-2} and ib_6, Rhegmatocytes, cb, oenocytoid, all from a young fifth instar; ib_{3-5}, rhegmatocytes from female adult $4\frac{1}{2}$–21 hours old.

F, k, Plastid from female adult $4\frac{1}{2}$–21 hours old; d_1, plasmatocyte from a female pupa 1 day old; d_2, plasmatocyte, dd, microplasmatocyte, l_1, mitotic figure (metaphase), l_2, mitotic figure (telophase), all from a fifth instar.

G, m, Adipose cell; n, nucleus; d, plasmatocyte; gc, paleocystocyte; all from a male pupa 24–48 hours old.

A

B

C

PLATE IV

Blood Picture of the Southern Armyworm *(Prodenia eridania)*

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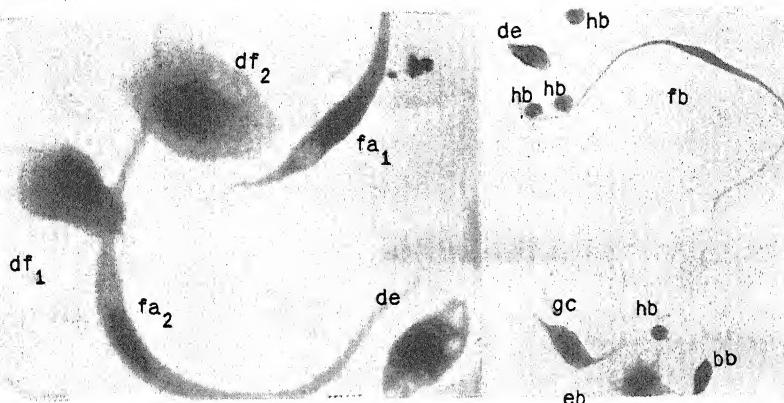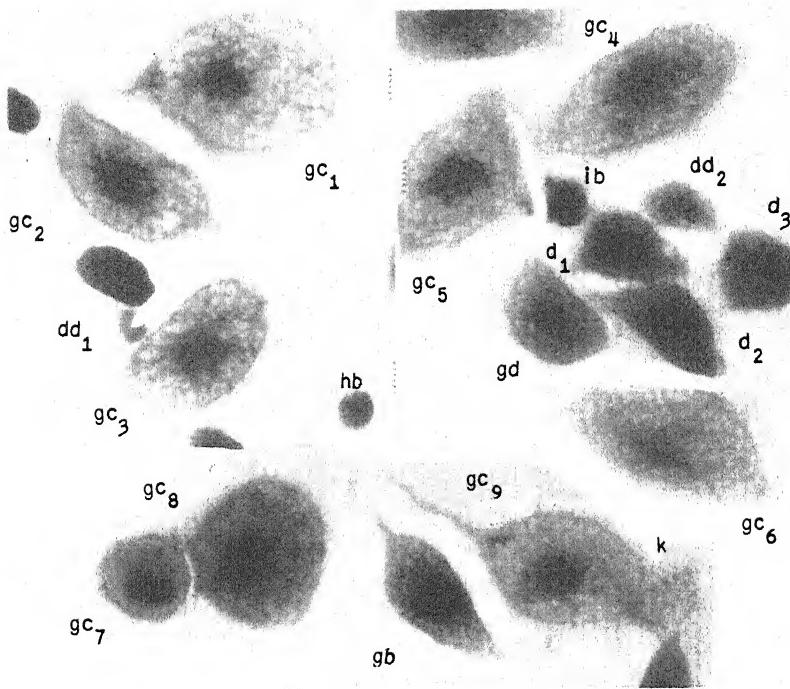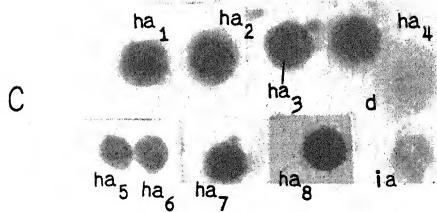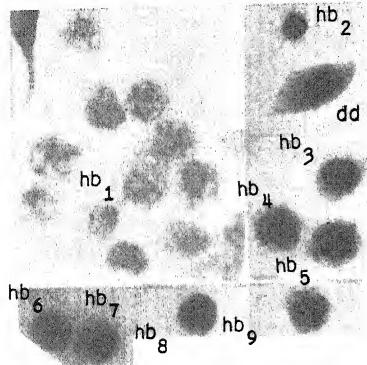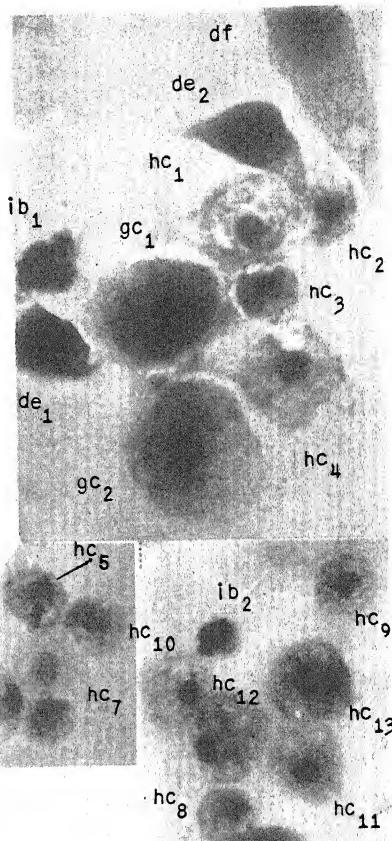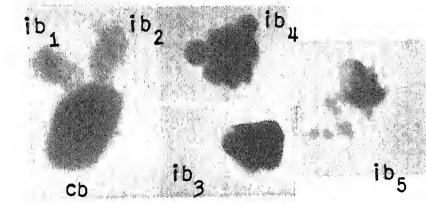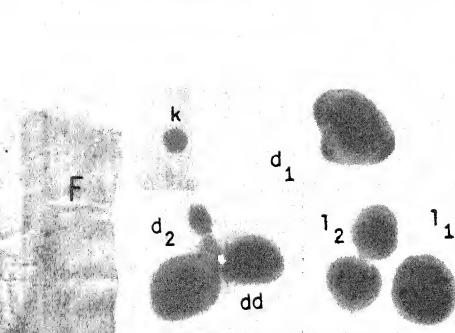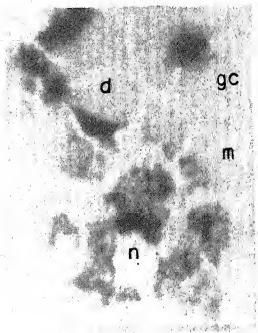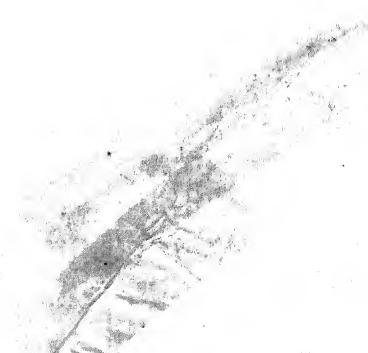
HYBRIDIZATION AND GENETICS IN USTILAGO HORDEI AND U. NIGRA [1]

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INTRODUCTION

It is well known that new strains of fungi pathogenic on plants may result from hybridization of existing species or races. Such strains are known particularly in the rusts and in certain smuts. Although it has been shown that certain species of the genus *Ustilago* on barley (*Hordeum vulgare* L.) may hybridize, it has not been clearly demonstrated that new physiologic races may result. To gain further information on this phase was the purpose of the present investigation.

There are at least three species of the genus *Ustilago* occurring commonly on barley. As early as 1888, investigators (*6, 20*) [3] recognized two kinds of smut on barley: Covered smut, caused by *Ustilago hordei* (Pers.) Lagerh., and loose smut, caused by *U. nuda* (Jens.) Rostr. In the head smutted with *U. hordei*, the chlamydospores remain enclosed for a time by a membrane; the spore walls are smooth; and the spores normally germinate by producing a promycelium that bears sporidia. In the head smutted with *U. nuda*, the spores are not enclosed long in a membrane but are liberated soon after the heading of the barley. The spores are echinulate and germinate by forming non-sporidia-bearing promycelia. *U. hordei* infects the very young seedlings, whereas *U. nuda* infects the young immature ovary during or soon after anthesis. In 1894, Biedenkopf (*5*) inadequately described what he considered a third species, *U. medians* Bied., in which he noted both types of spore germination described above.

More recently, investigators of barley loose smut in the United States have obtained unexpected results. For example, the long-accepted belief that loose smut of barley is controlled only by the hot-water seed treatment was brought into question when certain workers (*19, 21, 35*) obtained partial to complete control of loose smut with the formaldehyde seed treatment. Furthermore, Tisdale et al. (*36*) obtained loose smut infection of barley grown from seed superficially inoculated with what was interpreted to be *U. nuda*, whereas the original work (*10*) with this species had indicated blossom inoculation as the only means of obtaining infection. The possibility that dif-

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ferent physiologic races of *U. nuda* might enter the host by different means was suggested by Tisdale and Tapke (*35*) as an explanation of such results. The correct interpretation of these and similar results obtained by others in seed-treatment experiments was developed by Tapke (*27*) in 1932, when he demonstrated the existence of a second loose smut of barley, which he described and named *U. nigra.* In this and subsequent studies (*29, 32, 33*) Tapke reported consistent differences between *U. nuda* and *U. nigra* in method of infection and response to control through seed treatment with surface disinfectants. These two species were shown to differ also in type of chlamydospore germination; the echinulate chlamydospores of *U. nigra* normally germinate by forming a short promycelium bearing four lateral sporidia. Tapke (*33*) has further established the significance of these observations as a basis for the specific designation of *U. nigra.* He has also pointed out that *U. medians* Bied. cannot be accepted as a valid species because it was doubtless erroneously based on a field mixture of *U. nuda* and a sporidia-producing smut that may have been similar to *U. nigra.*

Only a limited amount of work has been done on hybridization between species of barley smut. Ruttle (*24*), on the basis of her own investigations and those of others, divided the barley smut collections she was investigating into seven types, ranging from typical *Ustilago hordei* to typical *U. nuda.* She suggested the hybrid origin of some of the types she observed, but this was necessarily only a speculation as it is obviously impossible to obtain conclusive evidence of hybridization from miscellaneous field collections alone. Allison (*1*), who studied the hybridization of barley smut species, was unable to obtain hybrid spores by inoculating barley with haploid combinations of *U. nuda* and *U. hordei*, but he did succeed in hybridizing *U. hordei* with what he designated as *U. medians.*[4] The smutted heads produced by these hybrids were morphologically intermediate between the covered and loose types but tended toward the loose condition. Moreover, the size of the chlamydospores of these hybrids was intermediate, and the echinulate character of the spores was dominant over the smooth character. While there were some differences in degree of pathogenicity between the hybrids and their parents, these differences were not sufficiently definite to show that new physiologic races had resulted from the cross. Indications, however, were in that direction.

Hybrids between other species of the smut fungi have been extensively studied (*9, 15, 16, 17, 20, 38*). In connection with the studies on the barley smuts, it seemed desirable to study in some detail hybrids between *Ustilago hordei* and *U. nigra.* The studies included the following lines of investigation: (1) Spore germination of the parent species and hybrid populations under different environmental conditions; (2) spore measurements; (3) sporidial fusion and nuclear behavior; (4) sexual compatibility; (5) hybridization and segregation for certain characters; and (6) pathogenicity of hybrids and inbred lines and the symptoms caused by them on the host.

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[4] The barley smut fungus with dark chocolate brown to black spore mass and with echinulate chlamydospores that germinate by means of promycelium and sporidia (*1, p. 15*) has been shown by Tapke (*33*) to be *U. nigra.*

MATERIAL AND METHODS

Ustilago hordei race 6 (*31*) and *U. nigra* races 4 and 6 (*34*) were used in these tests. Chlamydospore material, which had been purified by collecting the spores and reinoculating the same barley varieties over a 3-year period, was supplied by V. F. Tapke. In addition, the parent material was selfed by the writer for five chlamydospore generations before it was used for hybridization studies. The material supplied by Dr. Tapke agreed with the descriptions of the two species. The *U. hordei* material consistently produced the covered type of smutted head on barley; the chlamydospores were smooth and, when germinated on 1.5-percent potato-dextrose agar at room temperature, formed promycelia with sporidia. Likewise consistently, both races of *U. nigra* produced the loose type of smutted head on barley; the chlamydospores were echinulate and, when germinated on 1.5-percent potato-dextrose agar at room temperature (*32*, *33*), formed promycelia with sporidia.

All cultures used were of monosporidial origin. Single sporidia were isolated according to the method described by Hanna (*12*), except that a Chambers micromanipulator was used to hold the isolating needle. Chlamydospores were placed on a 1.5-percent potato-dextrose agar drop on a cover glass. When they had germinated, the sporidia were isolated from the promycelium of individual chlamydospores and cultured separately. Each chlamydospore from which such isolations were made was given a letter and number, and the sporidia were numbered according to their respective positions on the promycelium, beginning at the apex. Thus, a culture labeled H61 originated from *Ustilago hordei* race 6 and from the sporidium taken from the tip of the promycelium. Similarly, N41 originated from *U. nigra* race 4 and from the tip sporidium. The compatibility of the sporidia from individual promycelia was determined by mixing the respective sporidial cultures in pairs in all possible combinations and spreading the mixtures on poured plates of water agar. The presence or absence of fusion of the sporidia was determined by microscopical examination. When fusion occurred, the sporidial lines were considered compatible, and vice versa. Subcultures of the budding sporidia 2 to 3 weeks old were used as the source of inoculum. The cultures were maintained on a medium consisting of 10 gm. of dextrose, 10 gm. of sucrose, 10 gm. of malt extract, the extract from 400 gm. of potatoes, and 18 gm. of agar-agar per liter of water.

Odessa barley (C. I.[5] 934), which is susceptible to the races of smut employed in this experiment, was used in all studies on progeny of segregating hybrids. For studies of range of pathogenicity, the varieties Nepal (C. I. 595), Lion (C. I. 923), and Himalaya (C. I. 1312) were used.

Tapke's (*28*) method, plus a partial vacuum, was used in making both chlamydospore and sporidial inoculations. The seed was first treated in formaldehyde (1 : 320) for 1½ hours, washed in water for 20 minutes, and allowed to dry 2 or 3 days before inoculation. Culture inoculum was prepared by making sporidial suspensions of compatible combinations of monosporidial lines in 10 cc. of distilled water just before inoculation. The sporidial suspension was then poured over the seed to be inoculated, and the mixture was evacuated in a

[5] C. I. refers to accession number of the Division of Cereal Crops and Diseases.

flask for 25 minutes. After evacuation the seed was planted immediately or was allowed to dry and was then stored until planted.

EXPERIMENTAL RESULTS

SPORE GERMINATION

The effects of environmental factors on the germination, on the type of promycelial structure produced, and on the development of primary and secondary sporidia of *Ustilago hordei* have been studied by various workers (*1, 18*). Similar studies for *U. nigra* have been reported by Tapke (*32, 33*). Hüttig (*18*) studied the effect of temperature on the chlamydospore germination of a number of species of smut. He found that *U. hordei* would germinate at 0° to 30° C. At 0°, 1°, and 30°, true sporidia failed to develop. No germination was obtained at 35°.

The writer made a study of the effect of various temperatures, from 4° to 36° C., on the percentage of chlamydospore germination in *Ustilago hordei*, *U. nigra*, and two F_2 hybrids between them. The chlamydospore walls of hybrid 1 were smooth, whereas those of hybrid 2 were echinulate. All the chlamydospores were collected and stored under comparable conditions until the time of the test and were then germinated on 1.5-percent potato-dextrose agar in Petri dishes. The results are given in table 1.

TABLE 1.—*Effect of temperature on the percentage germination of chlamydospores of Ustilago hordei, U. nigra, and 2 hybrids between them* [1]

Temperature (°C.)	Age of culture	Germination [2] of chlamydospores of—				
		U. hordei race 6	U. nigra race 4	U. nigra race 6	Hybrid No. 1	Hybrid No. 2
	Hours	Percent	Percent	Percent	Percent	Percent
4	8	0	0	0	0	0
	36	43	49	0	0	0
	72	83	67	22	5	1
8	8	0	0	0	0	0
	36	44	47	5	1	20
	72	------	49	41	45	------
12	8	20	0	0	0	0
	36	83	21	12	10	30
	72	------	------	------	------	------
16	8	83	0	4	0	1
	36	------	50	------	------	------
	72	------	------	------	------	------
20	8	76	13	16	1	3
	36	------	------	------	------	------
	72	------	------	------	------	------
24	8	80	37	16	26	28
	36	------	------	------	------	------
	72	------	------	------	------	------
28	8	85	45	30	30	30
	36	------	------	------	------	------
	72	------	------	------	------	------
32	8	74	5	2	1	0
	36	82	25	27	17	2
	72	82	25	30	17	2
36	8	0	0	0	0	0
	36	50	0	0	0	0
	72	50	0	0	0	0

[1] Blanks in the table indicate that so many secondary sporidia and infection hyphae had developed that it was impossible to make germination counts.
[2] Percentages are averages of 2 replications.

In general, the temperature requirements for germination of the two species and the hybrids were somewhat similar. At 4° C., *Ustilago hordei* started to germinate within 24 hours, whereas *U. nigra* (N4, N6)

germinated more slowly. At this temperature, *U. hordei* produced an abundance of both primary and secondary sporidia, whereas *U. nigra* produced only a small number of primary sporidia and no secondary sporidia. The spores of the hybrids failed to germinate until they had been in culture for 72 hours, and then only a small percentage of the spores germinated and only a few sporidia were produced. At 4°, the percentage germination of the five cultures differed widely.

At 8° C., germination and development of the two species did not differ greatly from those at 4°. The two hybrids started germinating at 36 hours, instead of 72 hours as they did at 4°, but no infection hyphae were observed. The large increase in the percentage germination in the two hybrids at 8°, as compared with 4°, was the outstanding difference between the germinations at these two temperatures.

At 12° C., fusion of sporidia occurred only slightly in *Ustilago hordei* before 48 hours had elapsed and not at all in *U. nigra* or in the two hybrids. In *U. hordei*, the development of infection hyphae started after 48 hours at this temperature; and profuse development of secondary sporidia occurred in both *U. nigra* races and in the hybrids. In *U. hordei*, the percentage germination was not markedly different at 4°, 8°, and 12°. In the *U. nigra* races and also in the two hybrids, percentage germination at 12° and at 8° did not differ very greatly. At 16°, germination occurred within 8 hours in *U. hordei*, *U. nigra* race 6, and hybrid 2. Profuse development of secondary sporidia occurred in all cultures within 24 hours, and at the end of 36 hours sporidial fusion had taken place, with evidence of profuse development of infection hyphae.

At 20° C., a few primary sporidia were showing in *Ustilago hordei* at the end of 8 hours; whereas, in the *U. nigra* races and the hybrids, the promycelium was only one-third to three-fourths developed at that time. At the end of 24 hours, at 20°, the development of secondary sporidia was so profuse that it was impossible to determine the percentage of germination. After 72 hours at 20°, there was profuse development of infection hyphae in all of the cultures. They were most abundant, however, in the cultures of *U. hordei*. In *U. hordei* and *U. nigra*, there was little noticeable difference in growth and development at 20° and 24°. In the hybrids, however, the percentage germination was distinctly higher at 24° than at 20°. In *U. hordei*, at 28°, there was profuse development of secondary sporidia and a few infection hyphae were showing at the end of 8 hours. In the *U. nigra* races and in the hybrids, the promycelia were one-half to fully developed at that time, and a few primary sporidia were produced in *U. nigra* race 6. Profuse development of infection hyphae occurred between 36 and 48 hours. At the end of 72 hours all cultures of both species and hybrids were one solid mat of infection hyphae.

At 32° C., the type of germination was of special interest. The promycelia were short, very knotty, and constricted and failed to produce sporidia. At the end of 48 hours these promycelia had started to disintegrate, and by the end of 72 hours they had completely disintegrated. Promycelial fragments were scattered over the medium as though the promycelia had exploded. No true sporidia were produced, and, with the exception of *Ustilago hordei*, the percentage

of germination was considerably lower. At 36°, spores of *U. hordei* germinated up to 50 percent, while those of *U. nigra* and the hybrids failed to germinate.

SPORE MEASUREMENTS

Measurements were made of 100 chlamydospores and 100 sporidia each of *Ustilago hordei, U. nigra*, and the hybrids between them. The results are given in table 2. It is evident that the size, both of chlamydospores and of sporidia of the hybrids, tends to be intermediate between those of the parents (fig. 1).

TABLE 2.—*Measurements of 100 chlamydospores and 100 sporidia each of Ustilago hordei race 6, U. nigra race 4, and F$_1$ and F$_2$ of hybrids between them*

Species or hybrid	Mean size of—	
	Chlamydospores	Sporidia
U. hordei race 6	7.8 × 8.1	5.8 × 9.4
U. nigra race 4	6.8 × 8.2	3.8 × 10.9
F$_1$ of hybrid No. 1	6.8 × 7.3	
F$_2$ of hybrid No. 1	7.1 × 7.8	4.2 × 9.5
F$_1$ of hybrid No. 2	6.6 × 7.2	
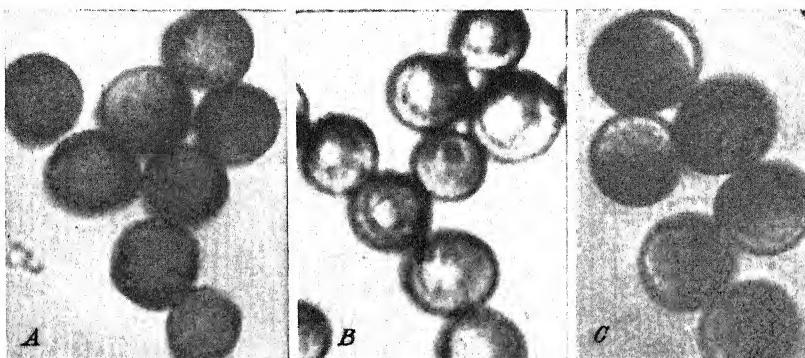
FIGURE 1.—Chlamydospores of (*A*) *Ustilago hordei*, (*B*) F$_1$ of *U. hordei* × *U. nigra*, and (*C*) *U. nigra*. × 1,800.

SPORIDIAL FUSION AND NUCLEAR BEHAVIOR

The various phases of genetics and cytology of the smut fungi have been studied by numerous workers. Hanna (*13*) and Stakman et al. (*26*) have adequately reviewed the progress of these investigations up to 1929.

Of the early workers, Kniep (*22*) was the first to report that fusion took place only between certain sporidia. He demonstrated the existence of two kinds of sporidia in *Ustilago violacea* (Pers.) Fuckl. and reported that they occurred in about equal numbers. Dickinson (*8*) later confirmed this with *U. levis* (Kell. and Sw.) Magn. and *U. hordei.* Hanna (*13*) and Holton (*15*) obtained infection of oats with crosses between *U. avenae* (Pers.) Jens. and *U. levis*, and found the hybrid spores to be echinulate. Flor (*9*) made crosses between *Tilletia tritici* (Bjerk.) Wint. and *T. levis* Kühn, and reported the hybrid

chlamydospores to be smooth.   Holton (*17*), on the other hand, work-
ing with the same species, found the hybrid spores to be reticulate.

A number of workers (*1, 2, 8, 11, 13, 15*) have described sporidial
and hyphal fusion of the various smut fungi.   Hüttig (*18*) and Allison
(*1*) have described in detail sporidial and other types of fusion and
the behavior of the dikaryophytic mycelium of *Ustilago hordei*.   How-
ever, no detailed study of sporidial fusion in *U. nigra* had been re-
ported previously.

In this study sporidial fusions have been observed between paired
sporidia of *Ustilago nigra* and *U. hordei* and between two races of
U. nigra.   Camera lucida drawings of individual sporidia and of
fused sporidia are shown in figure 2.   The fusion process that was
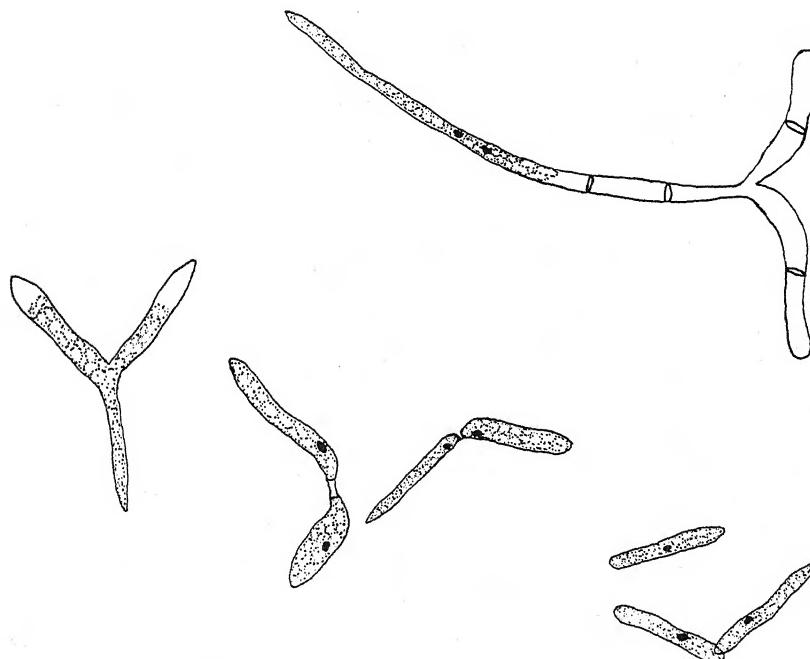
FIGURE 2.— Camera lucida drawings showing stages in the fusion of sporidia of
Ustilago nigra.   × 1,100.

observed was essentially the same as that described by Holton (*15*)
for *U. avenae* and *U. levis* and by Rodenhiser (*23*) for *Sphacelotheca
sorghi* (Link) Clinton and *S. cruenta* (Kühn) Potter.

In the present study, monosporidial lines of + and − sporidia of
Ustilago nigra were mixed and spread over poured plates of plain
water agar.   Observations were made to determine the time required
for these sporidia to fuse.   It was observed that fusion started within
4 hours and was most active within 4 to 6 hours, depending on the
temperature at which the cultures were kept.   All the sporidia ob-
served were uninucleate.

The various stages observed in the fusion process between com-
patible sporidia of *Ustilago nigra* are shown in figure 2.   Contrary to
observations reported by Holton (*15*) for *U. avenae*, that one sporidium

assumes an active role in the fusion process, all sporidia of *U. nigra* seemed to be equally active; however, it was evident that one sporidium became shorter and broader at the time fusion started. The first development observed was the appearance of a short projection at or near the tip of the sporidium. The projection increased in length until it came in contact with one from a sporidium of the opposite sex, and then fusion followed. Following fusion, the infection hypha was formed (fig. 2).

The fusion of the two sporidia initiates the dikaryophase. Following the completion of the fusion process, infection hyphae were produced. The hyphae in culture are aerial in habit and may not be observed in abundance until after 36 to 48 hours. After 72 hours, the cultures were a solid mass of aerial hyphae, whereas only an occasional aerial hypha appeared in the cultures of paired noncompatible lines. Within a short time after fusion, the protoplasm of the paired sporidia passed into the infection hyphae and migrated toward the tip as the hyphae increased in length. In stained preparations two nuclei that had migrated from the paired sporidia were seen within the cytoplasm. As the protoplasm progressed with the growth of the hyphae, cross walls appeared, cutting off the evacuated basal portions. An indefinite number of these empty cells, usually two or three, immediately behind the protoplasm remained turgid, while those back of that point collapsed and seemed to be without connection with the protoplasm.

The Bauch test, as used by Bauch (*2*, *3*, *4*) for the determination of sex differences between monosporidial lines of *Ustilago violacea*, *U. zeae* (Beckm.) Unger, and *U. scorzonerae* (Alb. and Schw.) Schroet., by Sleumer (*25*) for *U. zeae*, by Tyler (*37*) for *Sphacelotheca sorghi*, and by Rodenhiser (*23*) for *S. sorghi*, *S. cruenta*, and their hybrids, was not found satisfactory by the writer for determining sex differences in *U. nigra*. Even though fusion was profuse, fluffy white mycelium failed to develop, and the compatible combinations could be clearly identified only by the aid of a microscope. Holton (*15*) reported this test to be unreliable for *U. levis* and *U. avenae*.

Sexual compatibility in the various smut fungi has been studied by numerous workers. Christensen and Rodenhiser (*7*) reviewed the progress of these investigations up to 1940. Dickinson (*8*) was one of the first to report the segregation for sex factors to be on a 2 : 2 basis in *Ustilago levis* and *U. hordei*. Hanna and Popp (*14*) and Holton (*15*) found two sex groups in *U. levis* and . *Uavenae*. Allison (*1*) reported two sex groups for *U. medians* [6] and at least two arrangements of the + and − sporidia on a promycelium.

In the present study, experiments were conducted to determine the segregation of factors for sex in monosporidial lines from single promycelia of *Ustilago hordei* and *U. nigra*. Sets of 4 sporidia were isolated from the promycelium of each of 10 chlamydospores of *U. nigra* and from each of 8 chlamydospores of *U. hordei*. Each monosporidial line was cultured separately on the special malt agar described previously. For each species, the 4 monosporidial lines from each chlamydospore were paired with one another in all possible combinations. On the basis of sporidial fusion in culture, 5 of the 6 possible arrangements of the + and − sporidia on the promycelia were observed in *U. nigra* and all 6 arrangements were found in *U. hordei*. The detailed results

[6] See footnote 4, p. 42.

of the matings, given in table 3, show that the segregation of factors for sex in *U. nigra* occurred in the first division of the fusion nucleus in chlamydospores 3 and 4 and in the second division in the others.   In *U. hordei*, segregation occurred in the first division in chlamydospores 1 and 2 and in the second division in the others.   In both species the segregation for sex factors was in the ratio of 2 : 2, as reported by Allison (*1*) for *U. hordei*.

TABLE 3.—*Sex arrangement of sporidia (4 in each case) taken from promycelia produced by 10 germinating chlamydospores of Ustilago nigra and 8 chlamydospores of U. hordei, determined by pairing in culture all possible combinations of the individual sporidia*

Species from which sporidia were taken and sporidium No.	Segregation of sex factors in chlamydospore No. —									
	1	2	3	4	5	6	7	8	9	10
Ustilago nigra:										
1	−	−	−	+	−	+	+	−	+	−
2	+	+	−	+	+	+	+	+	−	+
3	−	+	+	−	+	+	+	+	+	−
4	+	−	+	−	+	−	−	+	−	+
Ustilago hordei:										
1	−	+	+	−	+	−	+	+	------	------
2	+	+	−	+	−	+	+	+	------	------
3	+	−	+	−	−	+	+	+	------	------
4	+	−	+	+	+	−	−	+	------	------

Fusion tests for compatibility grouping were made also between the four sporidial lines from one chlamydospore of *Ustilago hordei* race 6 in all possible combinations with those from one chlamydospore each of *U. nigra* race 4 and *U. nigra* race 6.   The results, given in table 4, indicate that there were two compatibility groups between *U. hordei* race 6 and each sporidium of the races of *U. nigra* tested.   Undoubtedly other compatibility groups would have been found if sporidial lines from additional chlamydospores from each species and race had been used.   It was noted, however, that there was a wide difference in the amount of fusion occurring in the various cultures of the compatible combinations.   In some of the cultures there was a small amount of sporidial fusion, while in others fusion was very profuse.   The percentage of fusion within a given culture was not determined.   However, where there was but little fusion between lines, there also was limited infection in the pathogenicity tests.

<div align="center">HYBRIDIZATION AND SEGREGATION</div>

A number of workers (*9, 14, 15, 16, 23, 26*) have reported the production of hybrid chlamydospores as a result of interspecific and intraspecific monosporidial crossing.   Allison (*1*) obtained hybrid chlamydospores in a cross between *Ustilago hordei* and *U. medians* [7] and reported that the F_1 produced smutted heads of intermediate type. Holton (*15*) and Hanna and Popp (*14*) obtained hybrid chlamydospores as a result of monosporidial crosses between *U. levis* and *U. avenae*.   Flor (*9*) reported hybrid chlamydospores as a result of interspecific monosporidial crosses between *Tilletia tritici* and *T. levis*. Rodenhiser (*23*) secured hybridization between *Sphacelotheca sorghi*

and *S. cruenta*, and Tyler and Shumway (*38*) between *S. sorghi* and *Sorosporium reilianum* (Kühn) McAlp.

TABLE 4.—*Compatibility (+) and noncompatibility (−) of 4 sporidial lines from a chlamydospore of Ustilago hordei race 6 with 4 sporidial lines from a chlamydospore of U. nigra race 4 and of U. nigra race 6, as determined by fusion*

U. hordei race 6, sporidium No.	U. nigra							
	Race 4, sporidium No.				Race 6, sporidium No.			
	1	2	3	4	1	2	3	4
1	+	+	−	−	−	+	+	−
2	−	−	+	+	+	+	−	+
3	+	+	−	+	+	−	−	+
4	−	−	+	+	+	−	−	+

In the present study, crosses were obtained between *Ustilago hordei* and *U. nigra* by inoculating seed of Odessa barley with combinations of monosporidial lines from each species. Chlamydospores from which the monosporidial cultures were obtained were the result of five chlamydospore generations of inbreeding. Inoculations were made also with intraspecific combinations of monosporidial lines from each species. Infection and chlamydospore production were obtained only from combinations that had produced fusion in culture. The intraspecific combinations produced chlamydospores that were characteristic of the species. The F_1 chlamydospores produced by the interspecific cross were echinulate, and the type of smutted heads produced by the *U. hordei* × *U. nigra* crosses ranged from almost typical *U. nigra* to the *U. hordei* type (fig. 3).
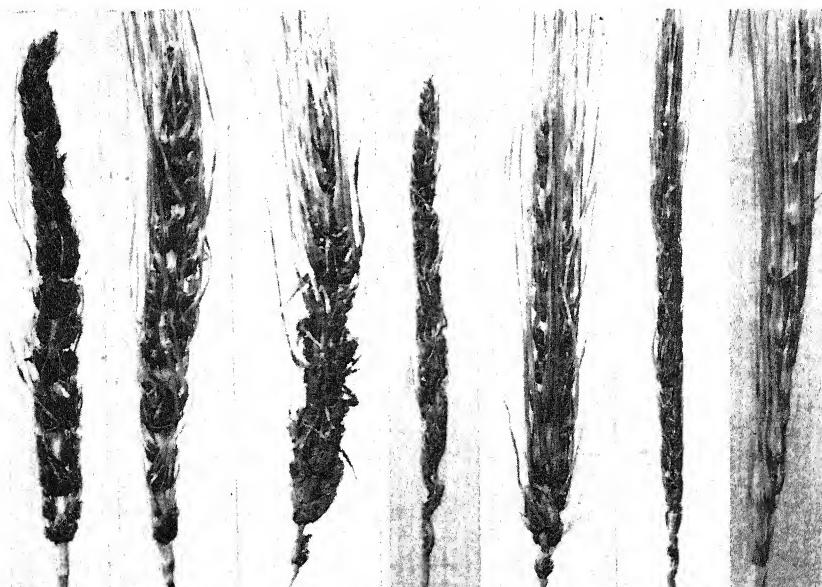
FIGURE 3.—F_1 smutted heads of Odessa barley, caused by inoculating the seed with compatible monosporidial lines from *Ustilago hordei* and *U. nigra*.

Studies were made to determine the manner in which factors for the echinulate-spore character and type of smutted head segregated. F_2 populations were obtained by inoculating Odessa barley with F_1 chlamydospores, and since the sporidia produced by hybrid spores are viable, inoculations also were made with combinations of mono-sporidial lines from F_1 hybrid spores and with backcrosses to the parent lines. The results of the inoculations with the F_1 chlamydo-spores are presented in table 5.

TABLE 5.—*Smutted heads of Odessa barley containing F_2 spores, either smooth or echinulate, following inoculation of seed with echinulate F_1 spores of the hybrids Ustilago hordei race 6 × U. nigra race 4 and U. hordei race 6 × U. nigra race 6.*

INOCULATED WITH U. HORDEI RACE 6 × U. NIGRA RACE 4

F_1 inoculum (spores echinulate)	Number of smutted barley heads containing F_2 spores—	
	Smooth	Echinulate
H61×N41	8	23
H61×N43	19	31
H62×N42	35	35
H62×N44	16	39
H63×N41	0	2
H63×N43	6	10
H64×N42	0	11
H64×N44	40	35
Total	124	186

INOCULATED WITH U. HORDEI RACE 6 × U. NIGRA RACE 6

H61×N61	7	12
H61×N63	0	11
H62×N62	1	1
H62×N64	0	2
H63×N61	6	10
H63×N63	6	5
H64×N62	10	19
H64×N64	1	2
Total	31	62

It will be noted from the data in table 5 that in the F_2 progeny there was no consistent segregation of smooth and echinulate characters according to the Mendelian 3 : 1 ratio. Only in the hybrid H61×N41 did the segregation approach this ratio. Similar results were obtained when inoculations were made with combinations of compatible monosporidial lines from individual promycelia of F_1 chlamydospores. As shown in table 5, most of the heads produced by the crosses had echinulate spores, but in two cases the number of heads with echinulate and the number with smooth spores were equal, and in two others the number of heads with smooth spores was the greater. Thus, in the majority of the crosses, echinulation was dominant over smoothness, as reported by Allison (*1*).

There is no obvious explanation of these results on the basis of the data at hand, unless the chlamydospores of the *Ustilago nigra* parent were heterozygous. This hardly seems possible, however, as the parent material was inbred for five chlamydospore generations. Moreover, two physiologic races of *U. nigra* were used, and it seems improbable that the chlamydospores of both races were heterozygous for spore markings.

The F_3 chlamydospore generation gave results similar to the F_2, except that in no instance did any cross produce a larger proportion of heads with smooth spores than with echinulate spores.

Two sets of sporidia were isolated from individual promycelia of the echinulate chlamydospores of the F_1 hybrid and used to backcross on each parent.

Pairings were made in all possible combinations and inoculated on Odessa barley. The results secured in the first-generation backcrosses on *Ustilago hordei* and on *U. nigra* are presented in table 6.

TABLE 6.—*Smutted heads of Odessa barley containing first-generation backcross chlamydospores, either smooth or echinulate, following inoculation with the backcrosses Ustilago hordei race 6 × (U. hordei × U. nigra); U. nigra race 4 × (U. hordei × U. nigra); and U. nigra race 6 × (U. hordei × U. nigra)*

INOCULATED WITH U. HORDEI RACE 6 × (U. HORDEI × U. NIGRA)

Sporidium No. in—		Number of smutted barley heads showing spores—	
Selfed parent	Hybrid parent	Smooth	Echinulate
1	2	51	32
1	4	31	84
2	1	31	54
2	3	4	7
3	2	8	24
3	4	29	68
4	1	12	76
4	3	8	48
Total		174	393

INOCULATED WITH U. NIGRA RACE 4 × (U. HORDEI × U. NIGRA)

1	1	5	46
1	3	9	28
2	2	4	10
2	4	9	22
3	1	0	16
3	3	0	14
4	2	2	2
4	4	0	4
Total		29	142

INOCULATED WITH U. NIGRA RACE 6 × (U. HORDEI × U. NIGRA)

1	1	1	3
1	3	4	22
2	2	8	35
2	4	1	6
3	1	2	11
3	3	0	3
4	2	2	1
4	4	1	14
Total		19	95

The results of inoculation with these backcrosses (table 6) show that the segregation of the smooth and echinulate characters of the chlamydospores was not in a simple Mendelian ratio. The smutted-head types produced by the backcrosses on the *Ustilago hordei* parent had a tendency to resemble those produced by *U. hordei* (fig. 4). In some instances the spores were smooth; in others they were echinulate. In

the backcross on the *U. nigra* parent, however, the smutted-head types ranged from the true *U. nigra* type to the true *U. hordei* type, with all degrees of intermediate types (fig. 4). Echinulate spores were not always associated with the *U. nigra* type of smutted head, nor smooth spores with the *U. hordei* type.
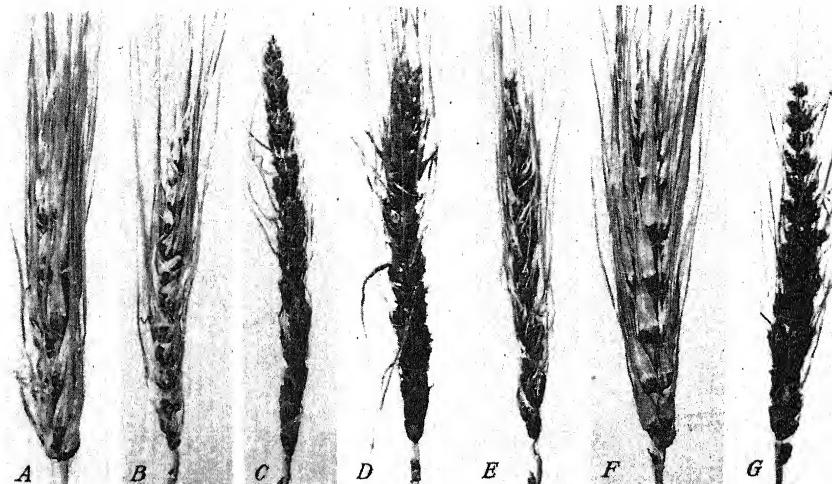
Figure 4.—Types of smutted heads of Odessa barley infected by (*A*) *Ustilago hordei* parent, (*B*) first-generation backcross on *U. hordei* parent, (*C* to *F*) first-generation backcross on *U. nigra*, and (*G*) *U. nigra* parent.

PATHOGENICITY

That new pathogenic races of smut fungi may arise through hybridization has been demonstrated by a number of investigators. Holton (*17*) in 1938 reported a new pathogenically distinct race resulting from a cross between *Tilletia tritici* and *T. levis*. Rodenhiser (*23*) also demonstrated that new pathogenic races result from crosses between *Sphacelotheca sorghi* and *S. cruenta*. Allison (*1*) found some evidence that new pathogenically distinct races of barley smut may result from hybridization of *Ustilago hordei* and *U. medians* [8] but they were not strikingly different. Therefore the present studies were undertaken to secure additional information along this line.

Inoculations with combinations of monosporidial lines were made on Odessa barley as previously described. The resulting F_1 chlamydospores, as well as chlamydospores from the parent lines, were used to inoculate separately seed of three varieties of barley, Nepal (C. I. 595), Lion (C. I. 923), and Himalaya (C. I. 1312). These varieties were among those used by Tapke (*29, 30*) for differentiating physiologic races of *U. hordei* and *U. nigra*. The inoculated seed was planted in the field in duplicate 5-foot rows, and the percentage of smut was determined by head counts. The duplicate rows averaged 200 heads per row. The results are presented as series 1 in table 7.

[8] See footnote 4, p. 42.

TABLE 7.—*Smutted heads of Nepal, Lion, and Himalaya barleys following seed inoculation separately with: (1) Selfed chlamydospores of Ustilago hordei H6, U. nigra N4, and U. nigra N6; (2) echinulate F1 chlamydospores of U. hordei H6 × U. nigra N6 and U. nigra N4 × U. nigra N6; and (3) echinulate and smooth F2 chlamydospores of U. hordei H6 × U. nigra N6 and echinulate chlamydospores of U. nigra N4 × U. nigra N6*

Inoculum				Smutted heads on—			Chlamydospore surface in heads of—			Smutted-head type[1] in Nepal, Lion, and Himalaya
Species and hybrid	Smutted-head type[1]	Chlamydospores		Nepal	Lion	Hima-laya	Nepal	Lion	Himalaya	
		Generation	Surface							

SERIES 1 (1939)

Species and hybrid	Smutted-head type[1]	Generation	Surface	Nepal (Pct.)	Lion (Pct.)	Himalaya (Pct.)	Nepal	Lion	Himalaya	Smutted-head type[1]
U. hordei H6	H	Selfed	Smooth	0	21.3	0	Smooth	Smooth	Smooth	H
U. nigra N4	N	do	Echinulate	0	37.0	0	Echinulate	Echinulate	Echinulate	N
U. nigra N6	N	do	do	32.0	9.0	17.0	do	do	do	N
U. hordei H6 × U. nigra N6	H, I, N	F1	do	4.0	3.0	4.0	Smooth or echinulate	Smooth or echinulate	Smooth or echinulate	H, I, N
U. nigra N4 × U. nigra N6	N	do	do	10.0	28.0	8.0	Echinulate	Echinulate	Echinulate	N

SERIES 2 (1940)

Species and hybrid	Smutted-head type[1]	Generation	Surface	Nepal (Pct.)	Lion (Pct.)	Himalaya (Pct.)	Nepal	Lion	Himalaya	Smutted-head type[1]
U. hordei H6	H	Selfed	Smooth	0	38.0	0	Smooth	Smooth	Smooth	H
U. nigra N4	N	do	Echinulate	0	36.0	0	Echinulate	Echinulate	Echinulate	N
U. nigra N6	N	do	do	37.5	6.0	25.5	do	do	do	N
U. hordei H6 × U. nigra N6	H, I, N	F2	Smooth	8.0	8.0	36.0	Smooth or echinulate	Smooth or echinulate	Smooth or echinulate	H, I, N
Do	H, I, N	do	Echinulate	9.0	7.0	40.0	do	do	Echinulate	H, I, N
U. nigra N4 × U. nigra N6	N	do	do	25.0	32.0	15.0	Echinulate	Echinulate	do	N

[1] H = U. hordei type; N = U. nigra type; I = intermediate.

It is of special interest to note that in the F_1 the interspecific hybrid *Ustilago hordei* H6 $\times$ *U. nigra* N6 tended to combine the pathogenic characteristics of its two parents, except virulence. That is, unlike *U. hordei* H6, it was able to attack all three test varieties. The percentages of heads smutted by this hybrid, however, were lower than those produced by either parent. Likewise, the intraspecific hybrid *U. nigra* N4 $\times$ *U. nigra* N6, unlike *U. nigra* N4, was able to attack all three test varieties. Thus, both of these hybrids differed from their parents in degree of pathogenicity. Furthermore, some of the heads smutted by the interspecific hybrid contained only smooth chlamydospores while others contained only echinulate ones. These two types of F_2 chlamydospores, and also the F_2 chlamydospores of the hybrid *U. nigra* N4 $\times$ *U. nigra* N6, were used separately to inoculate seed of Nepal, Lion, and Himalaya, which was planted in triplicate 5-foot rows. The percentage of smut was based on the total head counts of the triplicate rows. Each 5-foot row averaged 128 heads per row, making a total of 384 heads in a set. The results of the test are presented as series 2 in table 7.

The smut percentages in table 7 indicate that in both series of tests *Ustilago hordei* race 6 and *U. nigra* race 4 showed essentially the same degree of pathogenicity on the three test varieties; that is, neither of these species attacked Nepal or Himalaya and both attacked Lion about equally, whereas *U. nigra* race 6 attacked Nepal and Himalaya rather virulently and Lion only slightly.

In the F_2 of the interspecific hybrid (table 7, series 2), the smooth-spored and echinulate-spored segregates were similar in degree of pathogenicity. That is, both groups produced low percentages of smutted heads in Nepal and Lion and relatively high percentages in Himalaya. They differed, however, in these respects from both of their parents.

Likewise, in the F_2 the intraspecific hybrid *U. nigra* N4 $\times$ *U. nigra* N6 differed from its parents in degree of pathogenicity. That is, it produced rather high percentages of smutted heads on all three test varieties; whereas one parent produced a rather high percentage only on Lion and no infection on Nepal and Himalaya, and the other parent produced a low percentage on Lion and rather high percentages on Nepal and Himalaya.

In the F_2, both hybrids tended to combine the pathogenic characteristics of their parents in a manner somewhat similar to that in the F_1, except that the F_2 of the interspecific hybrid produced higher percentages of smutted heads on Himalaya than did either of its parents. Possibly this latter could be considered an example of transgressive segregation; that is, the virulence of the hybrid on Himalaya was greater than that of either parent on this variety.

Numerous microscopic examinations of hybrid chlamydospores showed that most smutted heads of the *Ustilago nigra* type had echinulate spores, whereas those of the *U. hordei* type had smooth or echinulate spores with approximately equal frequency.

DISCUSSION AND CONCLUSIONS

The parent chlamydospore material used in the present investigation was inbred for five chlamydospore generations, after being grown on the same barley varieties for three generations. That the inbred races of *Ustilago hordei* and *U. nigra* used were phenotypically pure is

shown by the fact that they produced smooth and echinulate chlamydospores, respectively, through the several successive generations. Evidence indicates, however, that all the races possessed different genotypes. For example, in the F_2 of the crosses H61×N41 and H61×N43 (table 5), the ratio of smutted heads with smooth chlamydospores to those with echinulate chlamydospores was 8:23 and 19:31, respectively. Similar examples are represented in the crosses H64× N42, H64×N44, H61×N61, and H61×N63. These data suggest that the parental material could have been (1) heterogenous, (2) heterozygous for one or more pairs of factors, or (3) heterogenous and heterozygous for morphological characters. In the F_2 of the crosses H62 and H64 with N42, H62 was more prepotent for the smooth-spore character than was H64. When H62 and H64 were crossed with N44, however, the reverse was true, indicating that factors governing spore markings interacted differently in different combinations.

The F_1 spores were echinulate, ranging from very minute to very distinct echinulation. Although Allison (*1*) reported a 3:1 ratio of echinulate spores to smooth spores in the F_2 from a cross between *Ustilago hordei* and *U. medians*,[9] no evidence of such a ratio was secured in the present studies of F_2 progeny of the cross *U. hordei* × *U. nigra*. The morphology of the heads smutted by the F_1 ranged from the *U. hordei* to the *U. nigra* type rather than being uniformly intermediate in type.

When smooth and echinulate F_2 chlamydospores were used separately as inoculum on Odessa barley, the percentage of smutted heads with smooth spores in the F_3 was practically the same from each lot. This suggests the possibility of some type of an inhibitor active in the F_3. On the other hand, when smooth F_2 chlamydospores of the same cross were used as inoculum on Himalaya, all of the F_3 spores were smooth. This suggests the possibility of a screening out of certain combinations in the F_3, due perhaps to differences in virulence of fused sporidial pairs from the smooth or echinulate spores or to differences in the relative resistance of the two varieties of barley.

It is apparent from the results presented that new physiologic races may result through hybridization of *Ustilago nigra* and *U. hordei*. Fertile hybrids were easily obtained from both interspecific and intraspecific crosses. In the tests on degree of pathogenicity, the hybrids in some cases were more virulent on Himalaya than was either parent. This was true particularly in the F_2 segregates for the smooth and echinulate character of spores. In the cross between the two races of *U. nigra*, there was evidence of a new race from the rather high percentage of infection on all three test varieties inoculated with F_2 chlamydospores.

SUMMARY

The optimum temperature for chlamydospore germination of *Ustilago nigra* and *U. hordei* ranged from 24° to 28° C. Chlamydospores of *U. hordei* germinated over a wider temperature range than did those of *U. nigra*. At 36°, *U. nigra* failed to germinate.

Ustilago hordei hybridized readily with *U. nigra*, as was evidenced by sporidial fusion in culture and by the production of hybrid chlamydospores in the host plants.

Interspecific crosses and backcrosses failed to show simple Mendelian inheritance of chlamydospore markings or pathogenic properties. Nor did the type of smutted head segregate in any such ratio.

The smutted heads produced from a monosporidial interspecific cross ranged in the F_1 from almost typical *Ustilago nigra* to the *U. hordei* type.

The backcrosses of the interspecific hybrids on the *Ustilago hordei* parent tended to produce smutted heads of the *U. hordei* type. In contrast to this, the backcross of the interspecific hybrid on the *U. nigra* parent produced all types of smutted heads, ranging from the true *U. nigra* type to the true *U. hordei* type.

In the F_2 of the interspecific hybrid there was some correlation between the chlamydospore markings and the type of smutted head. Most smutted heads of the *Ustilago nigra* type had echinulate spores, whereas the smutted heads of the *U. hordei* type had smooth or echinulate spores with approximately equal frequency.

Evidence was secured indicating that the range of virulence of *Ustilago nigra* and *U. hordei* may be combined through hybridization, thus forming new physiologic races.

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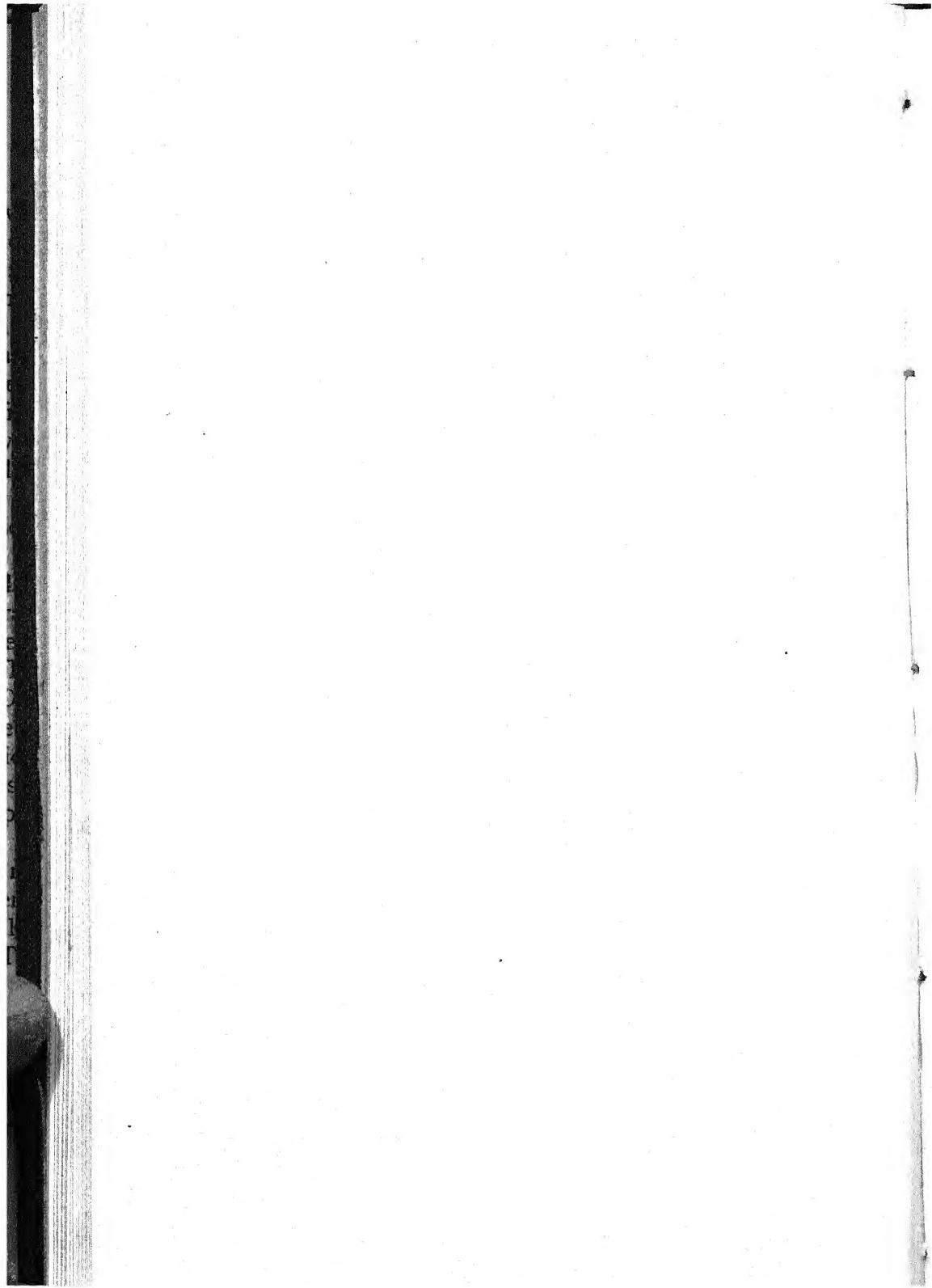
THE LOCATION AND STATE OF ROTENONE IN THE ROOT OF DERRIS ELLIPTICA[1]

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INTRODUCTION

Rotenone and its congeners have come into increasingly widespread usage as the active constituents of a large number of insecticides. A very extensive literature on rotenone and the rotenoids has been amassed; yet very little attention has been given to the histology of tissues containing these compounds in the parent plants (*Derris* spp., especially *D. elliptica* Benth.).[3]

Even though rotenone possesses a water solubility of only 15,000 micrograms per liter at 100° C. (*21*)[4][5], the total water extracts of the roots of plants of *Derris* spp. have, for a long time, been used as fish poisons in the Dutch East Indies and in South America. Van der Laan (*37*), Roark (*30*), Smith, Livengood, and Roberts (*33*), and others have reported that many insects also are very sensitive to the total water extracts from roots of *Derris* spp.

As early as 1919, McIndoo, Sievers, and Abbott (*25*) demonstrated that aqueous preparations containing derris act both as contact insecticides and as stomach poisons. However, a recent trend has been to use insecticidal sprays in which rotenone is dissolved in an oil. Such solvents of rotenone as heavy petroleum oils may cause serious injury to the twigs and leaves of the plant and to its fruits, from physical effects alone,[6] including, for example, the smothering effect of the oil and the adverse effect of the presence of oil in the leaf tissues on the normal physiological functions of those tissues.

While total water extracts of derris roots have been used as fish poisons and insecticides, the relative insolubility of rotenone in water suggested the advisability of reinvestigation of the location and state

[3] The genus *Derris* is a large one comprising many species of tropical woody vines (family Leguminosae, subfamily Papilionaceae, tribe Dalbergieae), particularly abundant in the Old World.

[4] Italic numbers in parentheses refer to Literature Cited, p. 77.

[5] When this investigation was undertaken, the only reference readily available to the authors on the solubility of rotenone in water was that of Jones and Love (*21*). Their value for the solubility of pure rotenone in water was, the writers believed, too low to account for the high toxicity of aqueous extracts of derris roots. It seemed, therefore, that derris resins might exist in the plant in some state permitting greater solubility in water than pure, chemically isolated rotenone.

Since the completion of the present work the data of Frear (*6 p. 80*) on the solubility of pure, chemically isolated rotenone in water at temperatures occurring in nature (i. e., 160 micrograms per liter at 25° C.) have become available. From this work and the work of Hamilton (*14*) it was evident that the solubility of pure rotenone in water was sufficiently high to account for its toxic action on most fish.

The rotenone content of the dry derris root of commerce averages about 5 percent, so that a solubility of 0.016 percent in water would preclude the existence of rotenone in a soluble state in the plant except under very special conditions. This paper shows that at least three special conditions are not satisfied in the plant and that rotenone is found in a resin which exists in the semi-solid state.

[6] Unpublished research, Division of Entomology, University of California Citrus Experiment Station, Riverside. These same physical phenomena seem to play an important role in the killing of the insect pests of plants sprayed with any material containing a heavy petroleum oil. (See also Kraiter (*24*) and Shepard (*31 pp. 204–211*).

of this substance in the plant cell. The present investigation was therefore initiated to obtain information which might form the basis for the development of a much-needed aqueous spray for the control of certain insect pests of citrus.

It was thought that rotenone might be contained in the living plant cell in a partially soluble form. Three possibilities seemed to exist: (a) That the rotenone might be dissolved in an ethereal oil, possibly one of a terpenoid nature; or (b) that it might be dissolved or suspended in the cell sap by means of an unknown solubilizer or mutual solvent; or (c) that it might exist as a glycosidelike compound.

Goodhue and Haller (*10*) found that rotenone-containing plants contained some unidentified sesquiterpenes, a finding which lent plausibility to hypothesis (a). Goodhue and Haller (*9*) and Worsley (*39*) demonstrated the presence of significant amounts of glucosides, similar to saponin, in ground derris root. The fact that the saponins are excellent emulsifying and dispersing agents gave credence to hypothesis (b). With reference to hypothesis (c), rupture of the cell wall in water would release the rotenoid as a glycoside, to form a true solution of the glycoside in water. Plants which synthesize glycosides usually possess cells containing enzymes capable of hydrolyzing these glycosides. Upon maceration of a piece of plant tissue, both glycosides and hydrolyzing enzymes are liberated, with the result that the effective concentration of the former decreases very rapidly. Such a mechanism might explain the failure to isolate glycosidelike rotenoid compounds from air-dried derris roots.

No data in support of hypothesis (b) are available, but the following mechanism of rotenone liberation might be postulated, namely, that the rotenone moiety would be deposited, not by hydrolysis, as in hypothesis (c), but by a change in phase relationships as the solvent in the plant was diluted extraneously with water.

EXPERIMENTS AND RESULTS

LOCATION OF THE ROTENONE-CONTAINING CELLS

Dried roots of *Derris elliptica* were boiled in distilled water for 30 minutes and then sectioned at 120μ under a steam jet on a sliding microtome. Fresh sections were made each day and kept between moist filter paper in a closed dish for use during the one day only, as moist derris [7] root is an excellent medium for mold growth. Unless otherwise stated, this procedure was the one used to prepare all sections from dried root throughout this study. In all such preparations, the water in which the samples were boiled acquired a pronounced milky opalescence; these colloidal extracts were used as the source of part of the material for certain tests to be described later in this paper.

A microscopical examination of sections mounted in water showed the xylem rays, pericycle parenchyma, phloem parenchyma, and xylem parenchyma to be very opaque. This opaque appearance was due to granules (or globules) contained in the cells. When treated with Durham's reagents (*36*, *20*), these opaque, amorphous bodies stained successively red, blue green, brown, and then black. The red coloration was produced by the nitric acid and appeared to

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[7] The term "derris," as used here and throughout the remainder of this article, applies specifically to *Derris elliptica;* unless otherwise specified, "derris resin" means the whole rotenone-containing resin (extractives) from *D. elliptica*. This usage refers only to those plants employed by the writers in their investigations.

be permanent if the sections were not treated further, but the addition of ammonia caused the other colors to appear in quick succession.

Sections immersed in 7-percent cupric acetate (Eckerson's test for resins) for 4 days exhibited the characteristic greenish copper-resin precipitate localized in the parenchymatous cells in the xylem rays and xylem, as shown in figure 1, *A*. Precipitates were found, also,
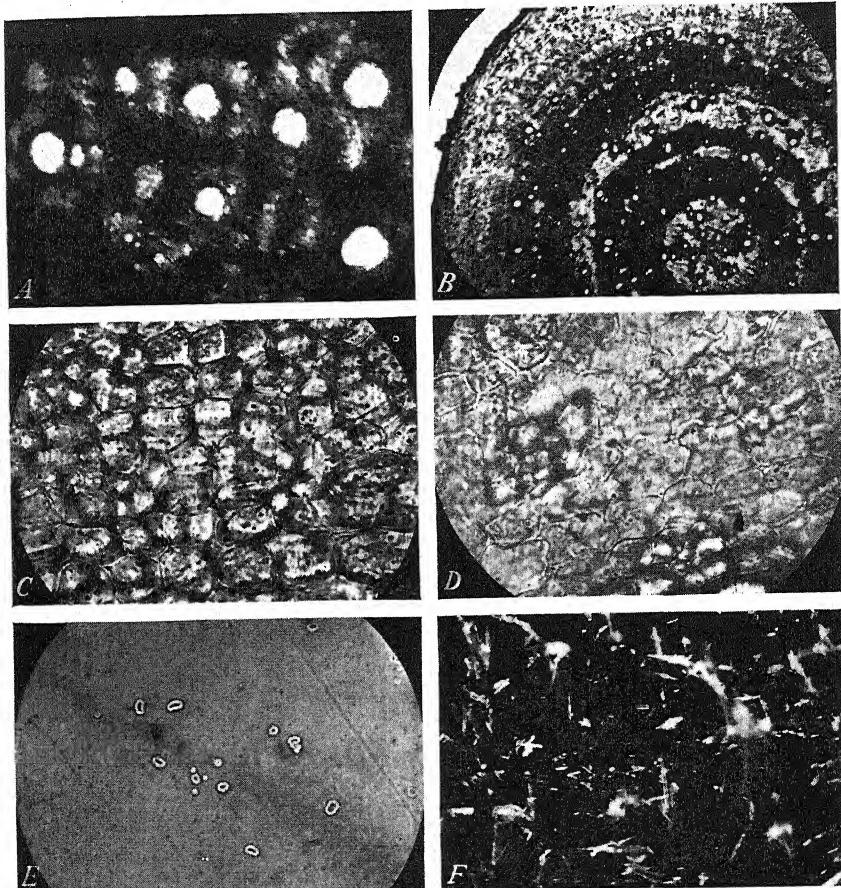
FIGURE 1.—Photomicrographs of sections of root of *Derris elliptica*, following different treatments. *A*, Transverse section of dried root after treatment with cupric acetate to show location of resin deposits. Dark spots in xylem rays and in xylem parenchyma in neighborhood of vessels indicate location of resin which contains rotenone; large, white circular areas are vessels; aggregates of small, light areas are xylem fibers, and grayish areas are xylem parenchyma containing starch. $\times$ 46. *B*, Transverse section, 8 mm. in diameter, stained with safranine, showing pentarch structure, the location of starch rings (dark areas), and lignification of parenchymatous cell walls; light rings are regions where rotenone is found. $\times$ 10. *C*, Transverse section, showing thick cell-wall structure, small vessels, and starch grains, as found in outer dark safranine-stained ring in *B*. $\times$ 217. *D*, Transverse section taken in light portion of dark ring found in *B*, showing thin cell-wall structure and fibers associated with thick-walled cells at edge of photomicrograph; many of the thin-walled cells contain rotenone. $\times$ 217. *E*, Starch grains (large) and resin particles (smallest particles in center and at left of photomicrograph) which diffused out of a section of the root into water. $\times$ 415. *F*, Mixture of crystalline osazones obtained from the aqueous extract of fresh roots. $\times$ 86.

in the phloem and pericycle parenchyma. No precipitate was found in vessels either large or small, or in the fibers.

Resins are of three types, classed as follows: I, common resins; II, oleo resins; and III, gum resins (*17, pp. 864–865*). As rotenone is oil-soluble, it seemed possible that Sudan II, which is an oil stain, might be used in the localizing of the derris resin containing rotenone. In order to characterize the type of staining reaction that might be expected, a number of different resins were tested. The results, presented in table 1 (reagent: Sudan II in 70-percent ethanol, 50-percent acetone solution, saturated), show that the heaviest staining reactions were given by the oleo resins, type II. On the basis of these tests, it is believed that derris resin probably belongs in type II.

TABLE 1.—*Reaction of type resins when treated with reagent Sudan II* [1]

Resin	Type [2]	Reaction
Benzoin	I	Negative.
Guaiacum	I	Few small red spots.
Shellac (liquid)	I	Red.[3]
Canada balsam	II	Do.
Turpentine	II	Do.
Cardolite 627 (phenolic resin)	II	Do.
Olibanum	III	Few small red particles.
Galbanum	III	Numerous red particles.
Derris resin (30-percent rotenone)	Unknown	Red to yellow.
Rosin	do	Small red spots.
Venetian turpentine	do	Negative.

[1] Sudan II in 70-percent ethanol, 50-percent acetone solution, saturated.
[2] Types: I, common resins; II, oleo resins; III, gum resins.
[3] This positive reaction was probably due to the solvent used.

When sections of derris root were treated with Sudan II and washed with 95-percent ethanol, many of the opaque cells stained deep red. These stained parenchymatous cells were found in the xylem, phloem, pericycle, and xylem rays, but vessels and fibers did not show the stain; thus the results of the Durham test [8] were substantiated.

To minimize the possibility of a positive Durham test for rotenone as a result of reaction with a resin rather than with certain constituents of a particular resin, several resins and pure rotenone were tested with concentrated nitric acid followed by 6 N ammonia solution. The results are collated in table 2.

TABLE 2.—*Reactions of type resins in the Durham test*

Resin	Type	Color reaction after treatment with—	
		HNO$_3$	HNO$_3$ followed by NH$_4$OH
Benzoin	I	Red brown	No change.
Guaiacum	I	Deep red brown	Do.
Shellac (liquid)	I	Red brown	Do.
Canada balsam	II	No color	Do.
Cardolite 627	II	Deep red brown	Do.
Turpentine	II	Red brown	Do.
Galbanum	III	Very deep red brown	Do.
Olibanum	III	Red brown	Do.
Derris resin	Unknown	Deep red brown	Blue green.
Rosin	do	Red brown	No change.
Rotenone [1]		Orange red	Intense blue green.
Tannic acid [1]		Deep red brown	Deep brown.
Venetian turpentine	Unknown	Red brown	No change.

[1] Tested for purposes of comparison and elimination.

[8] In the Durham test, substances such as deguelin and malaccol, which are similar to rotenone, give the color test for rotenone. Tephrosin gives no reaction, and toxicarol reacts but weakly.

Derris resin and rotenone alone gave a blue-green color when treatment with nitric acid was followed by treatment with ammonia. Accordingly, it was further substantiated that the Durham test is fairly specific for rotenone and structurally related compounds.

Apparently, the dark, nearly isodiametric parenchymatous cells, which average 70μ in diameter and $3.43 \times 10^5\ \mu^3$ in volume, and which are numerous in the xylem, phloem, and pericycle, and in the xylem rays, contain the rotenone. Under the polarizing microscope, these opaque bodies possess a very slight translucency and have an amorphous appearance; any power of double refraction they may possess is so slight as to be doubtfully discernible. When viewed with transmitted, nonpolarized light, these bodies had an opaque, amorphous appearance, as mentioned previously. When diffused light was employed, that is, when the microscope was placed in front of a window, the cells which contained the opaque bodies appeared sky blue in color, even when untreated with Durham's reagents. For this reason, it was found necessary to use great care in the interpretation of all the Durham tests reported in the present work. These opaque bodies did not stain with safranine, a fact indicating that they were not of an acidic nature.

As a further check on the opaque bodies found in the parenchymatous cells, fresh sections of derris roots were extracted for varying lengths of time (one-half minute to several days) with chloroform, diethyl ether, ethyl alcohol, and acetone. A few minutes in any of these solvents caused the disappearence of most of the opaque bodies, along with a clearing of the section. In each case, the extracted section was tested for the presence of rotenone by means of the Durham test, with only negative results; the corresponding extract was tested by means of the Goodhue (8) modification [9] of the Gross and Smith (11) color test, always with positive results. This fact indicated that the removal of rotenone from the sections accompanied the disappearance of the opaque bodies.

When the modified Gross and Smith color test was used as a stain in an attempt to stain the rotenone-containing cells directly, diffuse coloring was obtained because of the solvent action of the acetone necessary to the test. The only conclusion that could be drawn was that the color produced was most intense in and around the partially cleared opaque bodies in the parenchyma of the xylem, phloem, pericycle, and xylem rays.

Tapia Freses' (35) vanadium-pentoxide-sulfuric-acid test for rotenone was tried, also, as a microchemical test on fresh root sections, but with no success, as the tanninlike substances present in derris were found to give a positive reaction with this reagent. The partial hydrolysis of these tanninlike substances to tannic acid probably produced a purple color reaction with the vanadium reagent, for tannic acid itself reacted positively when tested.

When sections of fresh roots of *Derris elliptica* [10] were used the results of the preceding experiments were confirmed. The sections of fresh roots were prepared like those of the dried roots, except that a minimum amount of water was used to remove the section from the microtome blade. Fresh roots were not boiled or steamed. By

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[9] In the present tests it was found that if potassium nitrite is used instead of the sodium nitrite recommended by Goodhue (8), the final red color appears to remain unchanged for several days.

[10] These roots were kept packed in moist sphagnum moss while awaiting sectioning.

the use of a microinjection technique,[11] with the aid of a micromanipulator, it was found that the Durham test was positive for the fundamental central parenchyma, xylem rays, phloem parenchyma, pericycle parenchyma, and two concentric parenchymatous rings in the xylem. Vessels, fibers, and certain rings in the xylem, which alternated with the rotenone-containing cells, gave no reaction (fig. 1, *B*).

Since derris resin is quite mobile above 80° C., sections of fresh root were cemented with water glass to a slide maintained at 85° C. by means of an especially built, circulating-water-type slide heater (*13*). The attempt was made to puncture the walls of individual cells and to extract, by suction, the liquid, opaque, resinous, rotenone-containing bodies from the sections with a fine, hollow glass needle operated by a micromanipulator. The extracted rotenone-containing resin, thus isolated, was used for rotenone analysis. Little success was attained, but the opaque bodies in cut cells, when isolated, gave positive results in one test (450 opaque bodies isolated) and negative results in two tests (100 opaque bodies isolated for each of the latter tests). It is believed that the negative tests resulted from the isolation of too small an amount of material to respond to the test.

LOCATION OF THE STARCH-CONTAINING CELLS

When sections were flooded with an iodine-potassium-iodide reagent (0.3 gm. of I_2 and 1.5 gm. of KI, dissolved in distilled water to make 100 ml.), practically 80 percent of the cells stained blue. The presence of starch was thus shown in a large proportion of the parenchymatous cells, except those in the fundamental central parenchyma and in the phloem and pericycle, where only a very limited number of cells showed a positive starch reaction (fig. 1, *C*, *E*). The xylem rays stained dark; a few dark opaque cells were left unstained (fig. 1, *B*). Derris resin and pure rotenone did not react with the iodine reagent.

Other sections were stained with the Sudan II reagent; only a small number (about 10 percent) of scattered cells in the xylem, phloem, pericycle parenchyma, and xylem rays retained the stain (fig. 1, *D*). When the same sections were restained with the iodine reagent, the Sudan II-stained cells did not give a positive starch test, and the continuity of the original staining pattern was retained, an indication that starch and derris resin are not contained in the same cells. A number of large parenchyma cells dispersed in rings in the xylem did not stain with either reagent but remained transparent. These cells may have been cut in sectioning and thus may have lost either starch, rotenone, or contained tannin. Fibers and vessels also remained transparent.

Certain opaque cells in fresh sections became clear and translucent when flooded with acetone. Since acetone does not dissolve starch, the clearing action must have been due to the dissolution of the rotenone-containing resin. These same sections gave positive starch tests only in the uncleared regions, and the cleared areas did not give a test for rotenone when tested with Durham's reagent. These facts confirm Worsley and Nutman's (*41*) view in that they show that

[11] The microinjection needle was connected with an adjustable-height mercury reservoir, so that a very small drop of acid could be ejected onto a very small group of cells in the cross section of the root, the entire operation being viewed under the microscope. Colorations were observed both with and without the addition of ammonium hydroxide.

rotenone is present in cells which do not contain starch. Rotenone does not appear in cells which do contain starch.

Roots up to 6 mm. in diameter are lignified only in the vessel walls and in xylem and phloem fibers. Roots up to 11 mm. in diameter show lignification of the walls of the small and large vessels, the large vessels often being surrounded by many small vessels. Xylem, phloem, and pericycle fibers are also lignified, and the xylem, phloem, and pericycle cells which are parenchymatous but which have thickened walls (fig. 1, *C*) have lignified walls in large roots (above 6 mm. in diameter). Apparently, the cells having the thickened cell walls contain the starch and the thin-walled cells contain the rotenone (figs. 1, *D*, and 2).

The alternation of bands of starch-containing and rotenone-containing cells was noted by Moore and Jones (*26*). They also found that "low carbohydrate" plants, that is, those in the most active growing condition, contained the greatest number of thin-walled cells and the highest percentage of rotenone. These observations seem to correlate well with the presence of thin-walled, rotenone-containing cells and thick-walled, starch-containing cells observed in the writers' root preparations.

Subsequently, it was noted that when sections were treated with dilute nitric acid, as in the Durham test, the milky portions of the section, including the exudate, cleared very considerably. This clearing was similar to that produced by acetone, or by 95-percent ethyl alcohol, and probably was due to its solvent action on the derris resin.

Since nitric acid also probably hydrolyzes the starch in the Durham test, nitric acid, followed by ammonium hydroxide, was added to a water suspension of soluble starch in a macro-test: no color was produced, but the suspension was cleared. This test added further confirmation to the specificity of the Durham test.

<div align="center">LOCATION OF THE TANNIN-CONTAINING CELLS</div>

Three of the known constituents of derris resin, namely, malaccol, sumatrol, and toxicarol, contain free phenolic hydroxyl groups; the other four known constituents, namely, deguelin, elliptone, rotenone, and tephrosin, contain carbonyl groups capable of enolization. The production of a coloration with ferric chloride solution is typical of phenols and enols, but many of them do not give colors.

Since the aqueous extracts both of dried derris root and of rotenone-free dried derris root reacted with aqueous ferric chloride solution (10 percent by weight) to give the green color indicative of the presence of tannins, a series of experiments was carried out in an attempt to locate definite, tannin-bearing cells in root sections. Except for a barely perceptible darkening of the natural color of the solution, neither chemically pure rotenone nor 30-percent derris resin reacted with ferric chloride, either in aqueous suspension or in alcoholic solution. When sections of root were tested with ferric chloride, however, they showed the presence of very numerous darkly stained tannin bodies in the epidermis and a few scattered cells elsewhere. Although a solution of tannic acid itself reacted readily with the reagent, a water extract of the macerated root from which these sections came gave a negative test for tannins. It would thus seem

that the tannins present in the fresh root were not appreciably water-soluble. It could not be determined whether rotenone was present in these tannin-containing cells. The tannin test on root-cork particles was completely negative.

According to Nierenstein (*27*, *pp. 9–20*) and others, the color reaction between the tannins and iron salts is not specific, since many compounds having phenolic nuclei develop a color with iron chloride. Commercial saponin is distilled water and in the solid state likewise did not react with the ferric chloride reagent, even after 2 hours.

SUGARS AND OTHER REDUCING SUBSTANCES

Samples of the filtered aqueous extract (No. 30 Whatman filter paper in funnel) of fresh sections of root completely reduced boiling Fehling's solution after 20 to 30 minutes. When the filtered extract was made strongly acidic with hydrochloric acid, however, the Fehling's solution was completely reduced in 10 minutes. Another sample of the filtered acidified extract was boiled for 10 minutes, cooled, and made basic with ammonium hydroxide; this mixture completely reduced Fehling's solution in 2 minutes. A third sample of the filtered acidified aqueous extract was boiled for 10 minutes, cooled, and adjusted to pH 7 with dilute sodium hydroxide solution; this mixture, also, completely reduced Fehling's solution in 2 minutes.

The interpretation of these results is that the filtered aqueous extract of derris root, which contained rotenone, contained no uncombined simple reducing sugars but did contain at least one disaccharide, or trisaccharide, or a molecule with a sugar moiety. Acid hydrolysis liberated the reducing sugar. These facts do not eliminate the possibility that rotenone could exist as a glycosidelike compound, as indicated in hypothesis (c) (p. 62), although almost any plant examined will behave in this manner.

In order to characterize the sugars produced by acid or alkaline hydrolysis, the following tests were carried out. To 4-gm. samples of the (Seitz) filtered aqueous extract of ground derris root, each containing approximately 0.2 gm. of solids in solution, was added 0.4 gm. of phenylhydrazine hydrochloride, 0.6 gm. of crystalline sodium acetate, and 4 ml. of distilled water. The osazones were then prepared and precipitated in the manner of Shriner and Fuson (*32*, *pp. 37–38*) and of Huntress and Mulliken (*18*). The time required for the first precipitation of osazone material varied between 20 and 30 minutes. After filtration and drying at room temperature, the mixture of crystalline osazones resulting from this test (fig. 1, *F*) was compared by the time required for precipitation and microscopically with the pure osazones of arabinose, fructose, galactose, glucose, maltose, saccharose (sucrose), and xylose. Glucosazone was the only derivative definitely identified, although a few scattered crystals which may have been xylosazone were discernible. The paucity of these crystals precluded a positive identification, particularly since the osazone characterization for the "analysis" of a mixture of sugars is not too reliable, owing to the possible formation of mixed crystals, or the deformation of the normal habit of the individual crystals as a result of the presence of foreign substances in solution. With this one possible exception, however, the osazone precipitate presented the uniform crystalline appearance of glucosazone.

Glucose, fructose, mannose, cellobiose, maltose, and sucrose yield identical osazones. The three disaccharides mentioned (cellobiose, maltose, and sucrose) hydrolyze in acid solution to yield either glucose alone or glucose plus fructose. Since the aqueous extract of derris root was shown above to contain no simple reducing sugars, and since maltose and cellobiose are reducing disaccharides (reducing Fehling's solution readily), it may be that the sugar probably present as such in the extract was the disaccharide sucrose, which was hydrolyzed, through the acid treatment in the preparation of the osazones, into glucose and fructose.

Since rotenone and its congeners were removed from the aqueous extract with the Seitz filter and were not detected in the sugar solution, it was concluded that they did not exist as glycosidelike compounds in the plant, as suggested in hypothesis (c) (p. 62).

SAPONINS

A mixture of powdered or shredded derris root and water forms a remarkable amount of moderately stable foam when shaken. After Seitz filtration, the clear yellow to yellow-brown filtrate retains this ability to foam. In addition, derris dust causes sneezing, irritates the throat and nasal passages, and makes the eyes smart unpleasantly. These properties, in general, are characteristic of the saponins. In order to prove the presence of saponins or saponinlike substances, 100 gm. of powdered derris root was shaken with 500 ml. of distilled water, macerated for 1 hour, and then filtered through a Seitz filter (pad No. 6). This clear yellow filtrate, which contained no suspended matter, as indicated by the absence of the Tyndall effect, was used in all the following saponin tests.

To 9 ml. of this aqueous solution, which possessed a pH of 5.5 to 6.0 (nitrazine paper), was added 1 ml. of a light-medium grade petroleum spray oil; upon vigorous agitation, this mixture formed a very stable emulsion. A similar stable emulsion resulted when kerosene was substituted for the previously mentioned spray oil.

Although this filtrate did not precipitate when tested with 25 percent, or with saturated, ammonium sulfate solution it did yield a voluminous yellowish-brown precipitate with both saturated neutral lead acetate solution and saturated basic lead acetate solution. It reduced ammoniacal silver nitrate solution slowly. When 5 ml. of this aqueous extract was added to 10 ml. of a solution made by dissolving 5 gm. of potassium ferricyanide and 0.5 gm. of ferric chloride in 1 liter of distilled water, the resulting mixture turned greenish blue immediately, and then slowly deposited insoluble ferric ferrocyanide (Turnbull's blue) over a period of 2 hours. Another 5 ml. of the extract was evaporated to dryness, and the tan residue was divided into two portions. To one portion was added 2 drops of concentrated sulfuric acid, with the development of a reddish-purple color; to the other was added 2 drops of a solution composed of equal parts of concentrated sulfuric acid and ethyl alcohol containing a trace of ferric chloride, with the development of a red-brown color and a slight fluorescence.

To another 9 ml. of the aqueous extract was added 0.9 gm. of sodium chloride. After solution had occurred, 3 drops of fresh defibrinated beef blood was added. No hemolysis was apparent

after 2 hours, but some dissolution of the erythrocytes had occurred after 12 hours at room temperature. Chopra and Roy (3) found, however, that dilute (0.05-percent, or less) solutions of certain saponins and other hemolysins were completely inactivated by Seitz filtration. Another mixture of derris dust and distilled water was accordingly prepared and centrifuged until clear. The hemolysis test was repeated on a 9-ml. portion of this clear, supernatant liquid, with negative results, but again some hemolysis was apparent after 12 hours.

These experiments, in toto, indicate that there are one or more saponins present in derris roots. Ammonium sulfate does not precipitate these saponins from their aqueous solution, but they exhibit all the other characteristic reactions of their group. These particular saponins are slowly hemolytic. All the above experiments were checked as to type by means of parallel tests with a 0.1-percent aqueous solution of commercial saponin, with results as shown in table 3. Since saponins are definitely proved to be present in the root of *Derris elliptica*, it seems possible that these substances may exert a solubilizing action upon rotenone, and that hypothesis (b) (p. 62) may be given some credence.

Table 3.—*Comparison of saponin reactions of derris preparations and of commercial saponin preparations*

Test	Derris preparation	Saponin preparation
Foaming property	Positive	Positive.
Physiological effects [1]	do	Do.
Acidity of aqueous solution (nitrazine)	5.5–6.0	6.5–7.0.
Oil-emulsifying ability	Positive	Positive.
Precipitation by ammonium sulfate solution	Negative	Negative.
Precipitation by neutral lead acetate solution	Positive	Positive.
Precipitation by basic lead acetate solution	do	Do.
Reduction of ammoniacal silver nitrate solution	Weakly positive	Weakly positive.
Reduction of ferricyanide solution	Slowly positive	Positive.
Reaction with concentrated sulfuric acid	Purple-red color	Red color.
Reaction with concentrated sulfuric acid plus ferric chloride	Red brown (fluorescence)	Red brown (fluorescence).
Hemolytic power	Slowly positive	Positive.

[1] Causing throat irritation, sneezing, etc.

THE STATE OF DERRIS RESIN IN THE PLANT

When dried fragments of derris root were steeped in cold water for any length of time, it was noticed that the slightly acidic aqueous phase quickly acquired a milky appearance and exhibited the Tyndall effect; when fresh derris roots were cut or bruised, a milky exudate appeared immediately; when fresh derris fragments were placed in water, the aqueous phase acquired a milky appearance within a few minutes and exhibited a pronounced Tyndall effect. Under the low power of the microscope, both the exudate and the aqueous "milk" showed the presence of particles more or less equal in size, all of which seemed to polarize light under crossed Nicols. Under high power ($\times 744$), two sizes of particles were distinguishable: The larger ones (granules) were from 7μ to 38μ in diameter, and the smaller ones (globules) were from 0.8μ to 3.9μ in diameter, measured under the oil-immersion objective ($\times 910$). The larger particles were very much in the minority and were approximately 10 times the

size of the smaller ones. Only these large particles exhibited birefringence under high power. When this suspension was treated with iodine-potassium-iodide solution, the large particles stained dark blue and lost their birefringence. The addition of 6 N nitric acid to the "milk" slowly cleared it of all particles, as could be readily observed under the microscope by running small amounts of the acid under the cover glass with a pipette. Similar experiments with acetone dissolved the small particles and left the large particles. These tests indicate that the large particles are starch grains and that the small particles are resinous in nature. A photomicrograph of the particles is shown in figure 1, *E*. The small particles exhibited Brownian movement; the large ones did not.

Several samples of both dried and fresh derris root were thoroughly triturated and macerated, separately, with distilled water. After steeping for several days at room temperature, each sample was filtered, first through an ordinary qualitative filter paper to remove fragments of the secondary plant structure, and then through a Seitz filter (pad No. 6) with 25 pounds pressure and 4 cm. vacuum. The clear filtrates were straw yellow in color, were free from colloidal material, and did not exhibit the Tyndall effect. A portion of each filtrate was evaporated to dryness on the steam bath; the residues were tested for the presence of rotenone, both by the Durham test and by the modified Gross and Smith test, with completely negative results in each case. Other portions of unevaporated filtrate were combined (about 20 ml.) and extracted twice with 20-ml. portions of carbon tetrachloride. The combined carbon tetrachloride extracts were evaporated to dryness on the steam bath, 20 ml. of 95-percent ethyl alcohol was added, and the solution was again evaporated to dryness. Another 20 ml. of ethyl alcohol was added to the residue, and the evaporation was repeated. When dissolved in acetone and tested with the modified Gross and Smith reagents, the residue showed complete absence of rotenone and deguelin. Other portions of the filtrate were acidified with hydrochloric acid, evaporated to dryness, and tested for rotenone, with negative results.

The filter pad from each filtration showed a yellow-brown deposit matted in the fibers of the pad. This deposit gave a very strong starch test with iodine-potassium-iodine solution. The deposits from four pads were extracted for 30 minutes with acetone; the acetone extract showed a very high concentration of rotenone when tested by the modified Gross and Smith test, or by the Durham test after removal of the solvent. Another pad with residue, tested directly with Durham's reagents, showed a high rotenone content.

In another experiment, 10 gm. of fresh derris root was shredded into a Waring blendor and extracted with 400 ml. of distilled water for 3 minutes at 12,000 revolutions per minute . The resulting suspension was filtered through qualitative paper, and the filtrate was refiltered through the Seitz filter (pad No. 6). After this filter pad had been dried at room temperature, it was extracted at room temperature with 50 ml. of dry diethyl ether in the dark for 80 days. The ether was removed from the resulting clear extract, in vacuo, and a tan-colored semicrystalline residue, weighing 0.25 gm. (2.5 percent by weight), was left. This residue possessed the odor characteristic of derris resin; it softened at 50° C. and was very motile at 100°. Equal

quantities of this material and of commercial 30-percent derris resin were mixed and warmed slowly alongside a comparison tube of the residue; the contents of both tubes showed similar physical properties (viscosity and appearance) through a range of 0°–100°.

These results indicate that rotenone exists in conjunction with the other components of derris resin within the plant cells as discrete globules of rotenone-containing resin.

THE CHARACTERIZATION OF DERRIS RESIN AS AN OLEO RESIN

As was mentioned earlier, tests with an oil stain indicated that derris resin is an oleo resin. Accordingly, it should be possible to isolate an "oil" from derris resin.

Steam distillation of 300 gm. of a commercial derris resin (30-percent rotenone) from *Derris elliptica* yielded 12.5 gm. (2.8 percent by weight) of a clear, faintly yellow oil with a musty odor. Atmospheric distillation yielded about 11 gm. of cis-dichloroethylene and a small amount of an intense-yellow liquid residue. The dichloroethylene appeared by virtue of the fact that it is one of the solvents used commercially to extract the "resin" from rotenone-bearing roots; it was characterized here by its physical and chemical properties. Vacuum distillation of this liquid residue yielded a very small amount of a clear, colorless, evil-smelling liquid which boiled at 47°–50° C./27 mm. This viscous liquid contained no halogen and burned with a very smoky flame; it slowly reduced Schiff's reagent.

Steam distillation of 100 gm. of finely ground dried roots of *Derris elliptica* (6-percent rotenone) yielded traces of a colorless oil and a white waxy substance. The total distillate was extracted with ether, and this extract was dried over anhydrous magnesium sulfate. Approximately 0.1 gm. of an orange-yellow, oily material remained after removal of the solvent. This residue, which possessed the same evil odor as the residue of oil from the commercial derris resin mentioned above, contained no halogen, burned readily with a smoky flame, and slowly reduced Schiff's reagent.

The properties of these apparently identical oils from *Derris elliptica* suggest that they contain a terpenoid substance which possesses one or more carbonyl groups.

DISCUSSION AND CONCLUSIONS

Koolhaas (*23*), by determining chemically the rotenone content of bark and wood parts of derris root, found that there was generally a slightly larger rotenone content in the wood than in the bark, although the bark also contained a considerable amount of rotenone.

Georgi and Teik (*7*) confirmed these findings, both by determining the total ether extractives of bark and wood separately and by determining chemically the amount of rotenone in bark and wood; these determinations were based on dry weight. In addition, they found the proportion of dry weight of the bark and of the wood to the whole root. Their figures indicated, also, a little less rotenone or ether extractives in the bark than in the wood.

Several attempts have been made to find a distinct and characteristic microchemical test to distinguish a root rich in rotenone from one poor in rotenone. Van der Laan (*37*) found that a very sensitive color reaction[12] for solutions of rotenone, described by Jones and Smith (*22*),

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[12] Actually the Durham test. See footnote 8, p. 64.

could be applied also to microscopic sections of the root.   Sections of the roots were treated with 50-percent nitric acid and then flooded with 10-percent ammonia.   If rotenone was present, an evanescent blue-green color developed as soon as the ammonia had penetrated the cells.   The color lasted for only a few minutes; hence close, continuous observation under the microscope was necessary after the addition of ammonia.   By using this test, Van der Laan concluded that the active principle (rotenone) in derris root was localized in the parenchyma cells of the pith and medullary rays, in the parenchyma of the wood, and in the parenchyma of the bark.   No rotenone was found in the vessels of the wood or in the cork layers of the bark.

Though Buckley (*1*) found that there was apparently no toxicarol in varieties of *Derris elliptica*, and thus made the findings of Van der Laan more specific, Harper (*16*) found that malaccol, another rotenoid of *D. elliptica*, also reacted positively to the Durham test, with an intense blue color changing to green.   He postulated that this fact alone showed rings A, B, and C of malaccol to be the same as in rotenone itself.   Unless the rotenoids existed as a whole resin in these various portions of the plant, the nonspecificity of Durham's test for rotenone would render Van der Laan's findings even less concrete.   Results of the present investigation indicate, however, that rotenone and its congeners are elaborated concomitantly as discrete globules of whole derris resin in certain specific cells found in the xylem rays, in the xylem and phloem parenchyma, and in the pericycle.   The cells containing these globules of resin are most dense in two or more concentric rings in the xylem parenchyma.   The locations of the rotenone-containing regions are shown in figure 2.

By steam distillation of an extract of the shade-dried leaves of *Tephrosia vogelii*, Hanriot (*15*) isolated an oily liquid which had the reducing properties of an aldehyde.   Cahn, Phipers, and Boam (*2 pp. 204, 208, 209*) discussed methods of extracting "fats or rotenone" from *Derris* sp.   Goodhue and Haller (*10*) found the noncrystalline part of the extractives from *T. virginiana* to contain a considerable quantity of an oil composed mostly of sesquiterpenes.   In the present investigation, an oil which appears to contain at least one terpenelike compound with the reducing properties of an aldehyde was obtained from *D. elliptica*.

The globules of resin found in certain specific cells in *Derris elliptica* are probably in partial solution in a mixture of terpenelike compounds.   Thus, since derris resin seems to be an oleo resin, hypothesis (a) (p. 62) is substantiated.   The presence of saponins was shown to favor hypothesis (*b*).   We may therefore conclude that the resin exists within these specific cells in partial solution as an oleo resin, and that, upon destruction of the cell wall, the saponin influence tends to solubilize, or suspend, these globules of resin in any dispersing phase present.

In 1937, Spoon et al. (*34*) reported that discrete granules of starch could be discerned microscopically in samples of finely ground *Derris* spp.; they reported, furthermore, that the external appearance of these starch granules differed among species and might possibly be used as a criterion for identification.   In the same year, measurements of the cells of the woody structures of *Derris* spp. and of *Lonchocarpus* spp. were made and reported by Diakonoff (*4*).   In 1939, Guil-
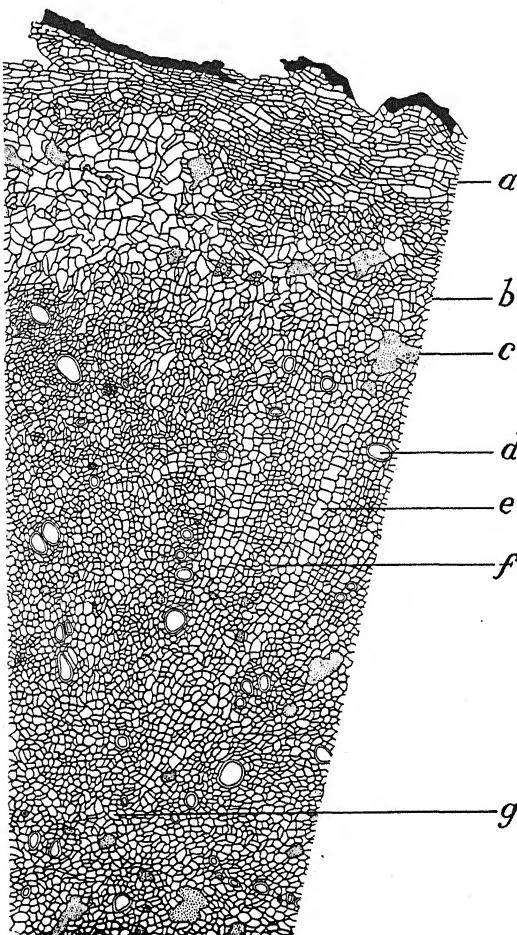
FIGURE 2.—Camera lucida drawing of a transverse section of a root of *Derris elliptica* 4 mm. in diameter. The outer periderm (*a*) and cork contained no rotenone; pericyclic parenchyma (*b*) contained rotenone (secondary phloem not distinguishable); pericyclic fibers, tannin cells (*c*), and secondary xylem vessels (*d*) (small vessels surrounding large vessels, not visible) contained no rotenone; medullary (xylem) ray (*e*) and xylem parenchyma (*f*) contained rotenone, but none was contained in the secondary xylem fibers; fundamental parenchyma (*g*) (center of section) contained rotenone. $\times$ 162.

laume and Hervé (*12*) reported that different parts of the same derris root do not contain the same quantity of rotenone, and that small roots are richer in rotenone than large roots.

In a more detailed study, Worsley and Nutman (*41*) reported that resin-containing cells could be distinguished in microscopic sections of derris root by means of the Durham test. They concluded that rotenone occurs first in small cell groups in the secondary cortex opposite the protoxylem when the plant is about 6 weeks old, and that it spreads gradually throughout the xylem parenchyma and cortex. They further stated that starch and rotenone do not occur

in the same cell, but rather in mutually exclusive groups, and that the cells which contain rotenone are apparently unspecialized morphologically. It will be seen that these findings are in accord with those of Van der Laan (*37*), previously mentioned.

Worsley and Nutman (*41*) and Van der Laan (*37*) were probably justified in basing their conclusions almost entirely upon their observations with the Durham color reaction, for Jones and Smith (*22*) had shown previously that this reaction was well established as a rapid qualitative test for rotenone and related compounds. The writers felt, however, that, since the reactions involved were obviously so violent chemically, any microscopic observations following a treatment with strong nitric acid and hydroxide ion (*19*, *29*) could not justifiably be considered as final proof.

In a more recent investigation, Worsley (*39*, *40*) found rotenone in almost every part of *Tephrosia vogelii*, including all the floral organs. In some organs it was present in normal cells, while in others it was present in morphologically distinct cells, often of considerable size. It was found in the seeds of *T. vogelii*, *T. candida*, *T. ehrenbergiana*, *T. toxicaria*, *Derris elliptica*, *D. malaccensis*, *D. uliginosa*, and *Mundulea sericea (suberosa)*. On germination of the seeds, rotenone either disappeared entirely or diminished greatly in amount, which "suggests that it is of some definite use to the plant." In all parts except the seed, but including the seed testa, rotenone remained undiminished after the plant or organ had withered or died. When kept in the dark until dead, small plants of *D. elliptica* showed a decrease in the rotenone content of their roots. Worsley (*39*), however, was unable to extract a rotenone-decomposing enzyme from the seeds of *Mundalea*. Considerable work on this problem of the role played by rotenone in the plant has been done at the Puerto Rico station by Moore and Jones (*26*).

Only one qualitative reference relative to the tannin content of *Derris* spp. has been found, namely, that of Power (*28*), who found water-soluble gallotannin in *D. uliginosa*. The present work has shown, however, that there are large quantities of tannins in *D. elliptica*, but that in the fresh root these tannins are not appreciably water-soluble. Aqueous ferric chloride solution applied to sections of roots showed the presence of very numerous darkly stained tannin bodies in the outer layer of the pericycle. The colorimetric tests available did not permit of a determination of whether rotenone occurs in these tannin cells also.

Goodhue and Haller (*9*) investigated some of the constituents of aqueous derris extracts qualitatively and reported that the filtered milky suspension so obtained responded to the Goodhue (*8*) modification of the Gross and Smith (*11*) colorimetric test for rotenone. Goodhue and Haller (*9*), furthermore, refer to the aqueous extract of derris root as the "milky suspension of resin." This reference appears to be the only recorded suggestion (cf. Wray (*42*)) that actual globules of whole derris resin are suspended in such an extract, and the authors cite no proof. Using dried root of *Derris* and *Lonchocarpus*, Diakonoff and Smulders (*5*) had demonstrated earlier that there was a direct correspondence between the content of dibutyl ether-extractable substances in these roots and the amount of material which could be extracted by water in the form of a white powder.

The present investigation has shown that the aqueous extract of dried derris roots and both the aqueous extract and the "sap" of fresh derris roots do indeed contain particles (globules) of whole derris resin in colloidal suspension, as shown by their isolation and indentification. These particles (globules) exhibit Brownian movement, range in size from 0.8μ to 3.9μ in diameter, and may be removed quantitatively from the suspension by filtering it through a Seitz filter (pad No. 6). Such suspensions also contain discrete particles (granules) of starch, as shown by standard selective color tests on isolated material. The starch grains range in size from 7μ to 38μ in diameter and may exhibit secondary Brownian movement from impact of the resin bodies. The aqueous "milk" from fresh root contains more starch than that from the dried root.

Goodhue and Haller (*9*), after investigating some of the water-soluble constituents of an aqueous extract of ground dried derris root, reported the presence of both glucose and levulose and saponinlike glucosides which induced foaming but which were nonhemolytic. Worsley (*38*) reported that he made unsuccessful attempts to isolate a saponin from *Mundulea*.

The writers have reinvestigated the sugar content of aqueous extracts of derris roots. Unconcentrated aqueous extracts reduced Fehling's solution after 20 to 30 minutes; acid hydrolysis of these extracts, followed by neutralization, reduced this reaction time to 2 minutes. Thus no uncombined, simple reducing sugars were present, but a disaccharide or a molecule with a sugar moiety was present, and the reducing sugars were liberated by the acid hydrolysis. These sugars were further characterized by the preparation of their mixed osazones; microscopic comparisons with pure specimens indicated the possible presence of fructose (levulose) and the presence of glucose from saccharose (sucrose), and probably xylose in the combined state. The indication from these tests was that the rotenoids do not exist in the plant sap as glycosides.

The unusual physical properties of aqueous extracts of finely ground derris root suggest the presence of large quantities of saponins. Aqueous extracts, when freed of all suspended material, exhibited the following characteristic tests for saponins: Foaming, emulsive, and reducing powers; precipitation with lead acetate; and specific color reactions. When converted into an isotonic salt solution, aqueous derris extract hemolyzed beef erythrocytes upon standing. Accordingly, there are slowly hemolyzing saponins present in derris root. While these substances may exert a synergistic effect upon the efficacy of rotenone as a toxicant, their effect on the state of rotenone in the plant cell could not be determined, although they may conceivably act as solubilizers or mutual solvents.

SUMMARY

Rotenone exists in situ in the form of whole derris resin (extractives) which occurs as discrete particles (globules) in certain cells in the xylem rays, xylem parenchyma, phloem parenchyma, and pericycle, and it does not occur in the vessels or in the fibers. Cells containing rotenone, in the resin form, are most numerous in the parenchyma of the phloem and of the xylem rays, being most dense in two concentric rings in the xylem.

Starch is also present in great quantities in rings in the xylem and phloem parenchyma, apparently alternating with rings of rotenone-containing parenchyma. No starch occurs in fibers or in vessels, and starch and whole derris resin are not contained in the same cells.

These particles (globules) of whole derris resin are in partial solution in an ethereal oil in situ. When the cell wall is broken the globules of resin and oil are suspended, probably by means of slowly hemolyzing saponins which were shown to be present, in the sap of the plant.

The milky sap exuding from freshly cut roots of *Derris elliptica*, and the milky aqueous extracts of either living or air-dried roots, are acidic suspensions (pH 5.5 to 6.0) containing these particles (globules) of whole resin, ranging from 0.8μ to 3.9μ in diameter, and particles (granules) of starch, ranging from 7μ to 38μ in diameter. The former particles exhibit Brownian movement, but both types of particles may be removed quantitatively from the aqueous suspension by passing it through a filter of low porosity.

Although aqueous extracts of derris root contain saccharose, which readily hydrolyzes to fructose and glucose, experimental evidence indicated that the rotenoids do not occur, in situ, in glycosidic combination.

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TOLERANCE OF LAMBS FOR BLACKSTRAP MOLASSES [1]

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INTRODUCTION

In normal years blackstrap molasses is a common constituent of commercial mixed sheep concentrates as well as of mixed feeds for other classes of livestock. When experiments showed that high levels of blackstrap molasses lowered the digestibility of some nutrients in the lamb ration (*1, 2*),[2] it was felt that further work should be done to find the critical point at which such reductions occur. Accordingly, two digestion trials were made to determine the tolerance of lambs for blackstrap molasses before a reduction occurred in the apparent digestion coefficients of the respective nutrients.

Previous work at the Oklahoma Station (*1, 2*) had shown that blackstrap molasses added to the ration of lambs at from 22.5- to 25.2-percent levels, depresses the digestibility of the fat in the ration. The apparent digestion coefficients of protein and nitrogen-free extract were also appreciably reduced, but the coefficient for the apparent digestibility of fiber was not significantly altered. In one trial (*2*) an addition of 10 percent of blackstrap molasses did not materially change the efficiency with which the lambs could digest the various nutrients in the ration.

Mitchell, Hamilton, and Haines (*6*) reported that additions of glucose reduce the ability of calves and lambs to digest protein and fiber and to utilize the energy of the ration. Hamilton (*3*) confirmed these results in studies with lambs. Johnson, Hamilton, Mitchell, and Robinson (*5*) showed that corn molasses lowers the digestion of protein and fiber in the ration of the lamb. Briggs and Heller (*2*) found that sucrose, corn sirup, as well as potassium salts, compounded to resemble the salts of commercial blackstrap molasses, produced noticeable changes in the digestibility of nutrients. An addition of the salt mixture depressed the digestion of all nutrients, but only the apparent digestion of crude fiber was lowered a significant amount. Sucrose materially reduced the apparent digestion of all nutrients except fiber, while corn sirup lowered the apparent digestion coefficients of all nutrients except nitrogen-free extract.

Optimum results with poultry have been secured in rations containing not more than 10 percent of blackstrap molasses. Upp (*10*) found rations containing 10 percent satisfactory for laying hens, but 15-percent levels were decidedly laxative. Ott, Boucher, and Knandel (*7*) presented evidence showing that a 4-percent level of molasses had a slightly stimulating effect on the early growth of chicks. Four- and six-percent levels increased feed consumption, but feed efficiency in producing gain was highest on a basal ration that contained no

molasses. These same workers (*8*) found that molasses could be used satisfactorily to the extent of 6 percent to replace yellow corn in the ration of laying hens.

PROCEDURE

Commercial blackstrap molasses was substituted for part of the corn in a basal ration consisting of corn, cottonseed meal, and alfalfa hay. Molasses was used at 5-, 10-, and 15-percent levels with 46, 90, and 136 gm. replacing an equal weight of corn in a basal ration containing 410 gm. of shelled yellow corn. The composition of the rations is given in table 1.

TABLE 1.—*Quantitative composition of the daily rations used in 2 digestion trials with lambs*

Feed	Grams of different feeds used in ration—			
	A	B	C	D
Alfalfa hay	454	454	454	454
Cottonseed meal	44	44	44	44
Corn	410	364	320	274
Blackstrap molasses	--------	46	90	136

The two trials were conducted on the same lambs and differed only in that the lambs were approximately 105 days older in the second study than in the first and the trials were carried out under different climatic conditions. The first trial was startted in midwinter; the second was conducted in late spring and early summer. The lambs used were the eight heaviest in a shipment of grade Rambouillet feeders from southwestern Texas. Their average weight was about 80 pounds at the beginning of the first trial and they gained approximately 0.1 pound daily during each trial.

The lambs spent a 10-day preliminary feeding period in a 5- by 4 foot pen and were then placed in a false-bottom metabolism cage for a 10-day collection period. The lambs were fed in open buckets in the pens and in a specially constructed feeder while in the cages; very little feed was lost in either feeder. One-half of the daily feed allowance was given in the morning and the remainder at night. The feeds were weighed on a scale sensitive to 0.1 gm.

The alfalfa hay was ground in a hammer mill and forced through a ¾-inch-mesh screen. The shelled corn was fed whole and the cottonseed meal was of the popular pebble-cake size. The blackstrap molasses was weighed, diluted with water, and added to the other feeds. The sugar determinations on the molasses showed the product to have 44.1 percent total sugar. The sucrose content was 29.0 percent, and 15.1 percent was invert sugar.

Feces were collected once each day and the collection for each lamb was dried at 90° C. for 24 hours. The collection was then weighed and sealed; at the end of the 10-day collection period the dried feces for each lamb were placed in a container, thoroughly mixed, and sampled for analysis.

TABLE 2.—*Percentage composition of feeds used in digestion trials with lambs*

Feed	Water	Protein [1]	Fat	Crude fiber	Ash	Nitrogen free extract
Alfalfa hay	10.97	17.70	1.69	29.98	7.49	32.17
Corn	11.88	8.89	3.19	1.95	1.46	72.63
Cottonseed meal	7.94	42.03	5.69	10.19	5.91	28.24
Blackstrap molasses	34.65	4.98			8.05	52.32

[1] N×6.25.

The chemical composition of the feeds used are given in table 2. The alfalfa hay was U. S. No. 2 grade; the corn was U. S. Sample grade. The same feeds were used in both trials.

RESULTS

Each of the eight lambs used was fed the four rations A, B, C, and D during both trial 1 and trial 2. The apparent digestion coefficients for the rations are given in table 3.

TABLE 3.—*Apparent digestibility (percent) of lamb rations* [1] *containing various levels of blackstrap molasses*

Lamb No.	Protein digestion coefficient for ration—				Fat digestion coefficient for ration—				Crude fiber digestion coefficient for ration—				Nitrogen-free extract digestion coefficient for ration—			
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Trial 1:																
409	74.8	76.8	74.7	70.1	75.4	74.0	65.5	66.7	56.2	57.5	57.5	54.6	87.2	88.0	87.7	85.9
393	73.3	69.3	72.8	72.9	70.0	62.0	66.5	57.3	53.3	50.4	61.4	62.8	85.9	83.9	87.4	87.3
330	74.9	73.2	73.5	73.8	73.8	73.1	70.2	63.1	54.4	56.9	57.5	58.2	86.0	86.8	86.6	85.8
374	77.0	75.3	74.7	74.1	71.5	72.7	56.4	61.3	61.8	56.6	60.7	60.5	89.1	87.9	87.3	87.3
366	73.2	74.6	72.9	74.4	69.4	70.6	75.0	62.6	56.5	57.3	54.5	53.1	87.0	86.8	85.3	85.6
308	75.4	74.3	74.4	74.4	62.5	65.6	68.6	65.9	60.1	60.6	57.2	55.5	87.0	86.1	87.0	86.1
324	74.7	76.2	75.8	70.7	71.5	69.5	72.5	68.9	55.2	58.3	54.0	54.3	88.0	87.1	85.6	83.4
345	72.7	71.4	73.3	71.9	63.2	64.0	65.8	68.7	51.3	59.2	54.8	56.1	86.8	87.6	87.9	86.5
Average	74.5	73.9	74.0	72.7	69.7	68.9	67.5	64.3	57.1	56.5	57.2	56.9	87.2	87.0	86.8	86.0
Trial 2:																
409	70.4	72.5	69.5	76.6	69.8	58.5	61.3	46.3	51.7	57.4	46.7	51.2	86.7	86.4	79.8	82.3
393	71.4	70.7	73.4	71.0	60.7	68.1	61.8	52.9	50.0	46.8	57.1	53.1	83.6	80.3	80.7	83.6
330	74.8	70.4	73.3	66.2	65.7	72.2	57.1	55.6	58.2	46.4	51.7	44.2	84.6	85.4	82.9	78.3
374	74.7	73.4	77.2	75.1	62.6	58.0	65.5	63.6	52.5	56.3	56.6	57.2	84.4	85.3	86.1	83.8
366	74.6	77.0	70.3	74.7	63.0	59.9	67.0	64.0	48.3	58.5	48.7	51.2	82.9	85.0	84.9	85.7
308	72.9	75.6	72.4	70.8	54.4	59.8	47.3	67.5	53.9	53.2	55.5	52.1	83.5	85.1	83.6	81.4
324	75.6	69.5	72.8	67.8	61.5	56.5	62.2	64.6	51.2	40.7	58.7	50.1	84.9	80.8	85.3	84.8
345	75.5	74.2	71.4	68.1	66.1	62.2	70.1	46.8	50.8	58.8	51.4	50.7	86.8	84.0	83.3	83.6
Average	73.7	72.9	72.5	71.4	64.2	61.9	61.5	57.7	52.1	52.3	53.3	52.9	84.7	84.0	83.3	82.9
Average, 2 trials	74.1	73.4	73.3	72.1	66.9	65.4	64.5	61.0	54.6	54.4	55.2	54.9	85.9	85.5	85.1	84.5

[1] A, basal; B, containing 5 percent molasses; C, 10 percent molasses; D, 15 percent molasses.

Table 4 shows the differences between the means of the 16 apparent digestion coefficients secured for each of the 4 rations. Snedecor's (*9*) test for significance was applied to the data of table 3 and the results are summarized in table 4. A value of $P=0.05$ was considered as the significant level; a value of $P=0.01$ was considered highly significant. The average coefficient secured for each ration in both the first and second periods are given in table 3, but they are not treated separately in the statistical analysis.

TABLE 4.—*Summary of the differences between means of apparent digestion coefficients of lamb rations and the significance of the differences* [1]

Rations compared [2]	Protein	Fat	Fiber [3]	Nitr gen-free extract
A and B	0.7	1.5	0.2	0.4
A and C	.8	*2.4	−.6	.8
A and D	*2.0	**5.9	−.3	1.4
B and C	.1	.9	−.8	.4
B and D	1.3	**4.4	−.5	1.0
C and D	1.2	**3.5	.3	.6

[1] *Significant at 0.05 point; **highly significant at 0.01 point; − not significant.
[2] A. Basal; B, containing 5 percent molasses; C, 10 percent molasses; D, 15 percent molasses.
[3] Minus sign indicates that digestion coefficient was less for check ration than for the one with which it was compared.

The feces of the lambs receiving ration D (15-percent molasses) were usually somewhat less dry and less well formed than those of lambs on the other three rations, but they were far from being as soft as those reported for lambs receiving a 25-percent level of molasses (*2*).

DISCUSSION

INFLUENCE OF 5-, 10-, AND 15-PERCENT LEVELS OF BLACKSTRAP MOLASSES ON PROTEIN DIGESTION

In this study each succeeding increase in the amount of blackstrap molasses further lowered the digestibility of the protein in the ration when molasses was substituted for corn in the basal ration of corn, cottonseed meal, and alfalfa hay. While each level of molasses tended to lower the efficiency of protein digestion, the 15-percent level was the only one at which the decrease was significant. The difference between the mean of the 16 coefficients was 2.0 percent whereas a difference of 2.1 percent would have been needed for the difference to be highly significant. The standard error of the difference between these means was 0.71. The lambs were less efficient in protein digestion in the second trial than they were in the first, and all lambs did not react alike to the different rations. This condition was handled statistically not only by analyzing the data for the difference between rations and within rations, but also by removing variations that occurred between the lambs from that within the lambs. In a single preliminary trial (*2*) on seven lambs a 10-percent level of molasses decreased digestibility of protein by 2.9 percent, but the difference was not significant.

It had previously been reported (*2*) that a 25-percent level of molasses lowered by 6.5 percent the digestibility of a ration containing similar basal feeds with almost identical digestibility. This difference was highly significant. It appears from these data on digestibility

that lambs tolerate a 5-percent level of molasses and digest protein with practically the same efficiency at the 10-percent level; higher levels hamper protein digestibility.

THE INFLUENCE OF 5-, 10-, AND 15-PERCENT LEVELS OF BLACKSTRAP MOLASSES ON FAT DIGESTION

In two previous studies (*1*, *2*) it was found that a level of blackstrap molasses approximating 25 percent of the ration caused a very marked reduction in the digestibility of the ether-soluble fraction. Sucrose and corn syrup likewise lowered the apparent digestion coefficients of fat. Neither potassium salts compounded to resemble those found in blackstrap molasses nor a 10-percent level of molasses produced a significant reduction, although in each case a depression was noted. Irwin, Weber, and Steenbock (*4*) have observed that large concentrations of either potassium chloride or calcium chloride decrease the digestion of fat in the rat.

There was a general decrease in the digestibility of the fat component of the ration at each level of blackstrap molasses and the decrease became more marked as the amount of molasses was increased. Molasses at the 5-percent level decreased fat digestion by 1.5 percent, but the difference was not great enough, considering the individual differences of the lambs, to be significant; at a 10-percent level the reduction was 2.4 percent and significant. A highly significant reduction resulted when molasses was used at a 15-percent level, the fat digestion of the ration being 5.9 percent less than that of the basal. The standard error of the difference was 1.06.

It is apparent that molasses cannot be used in excess of 5 percent without decreasing the ability of the lamb to digest the fat in the ration. The reduction observed at the 15-percent level, however, was less than that previously observed at higher levels (*1*, *2*).

INFLUENCE OF 5-, 10-, AND 15-PERCENT LEVELS OF BLACKSTRAP MOLASSES ON CRUDE FIBER DIGESTION

In this study, blackstrap molasses did not noticeably affect apparent crude fiber digestion coefficients at the three levels at which it was fed. There was a slight tendency for the coefficients to be higher at the 10-percent level than at the lower levels or at the 15-percent level. Fiber digestion was improved 0.6 percent when 10 percent of molasses replaced a like amount of corn in the basal ration. However, the standard error of the difference was 1.62 and the difference was not significant. In similar studies at this station (*1*, *2*) very slight reductions in fiber digestibility have followed the use of larger amounts of blackstrap molasses than were used in these studies, but the differences were not significant.

INFLUENCE OF 5-, 10-, AND 15-PERCENT LEVELS OF BLACKSTRAP MOLASSES ON NITROGEN-FREE-EXTRACT DIGESTION

The trend of the results obtained confirm observations of previous studies (*1*, *2*) that lambs do not make as ready use of that part of the ration classed as nitrogen-free extract when blackstrap molasses is included in the ration as they do a basal ration that contains no molasses. However, at the low levels of molasses used, the reductions were not significant. When molasses was fed at the 15-percent

level, it decreased the digestibility of nitrogen-free extract 1.4 percent; the standard error of this difference was 0.8 and the difference was not significant. Previous studies (*1, 2*) have shown that 25-percent levels of blackstrap molasses did lower nitrogen-free-extract digestion considerably while a 10-percent level did not.

SUMMARY

Eight wether lambs were used in two digestion trials to determine at what level of blackstrap-molasses feeding the apparent digestibility of the ration is altered. The molasses was substituted for corn at 5-, 10-, and 15-percent levels in a basal ration of corn, cottonseed meal, and alfalfa hay.

The ability of the lambs to digest protein was not influenced measurably except at the 15-percent level. Apparent fat digestion coefficients were reduced at both the 10- and 15-percent levels. Neither apparent fiber digestion coefficients nor those of nitrogen-free extract were influenced significantly by molasses at any of the levels used.

It appears from this study that blackstrap molasses can be used most effectively for lambs, so far as digestibility of the ration is concerned, when it constitutes not more than 5 percent of the ration. However, digestibility was not so greatly reduced at the 15-percent level but that it would often be economical to use the product at such a level in commercial lamb feeding.

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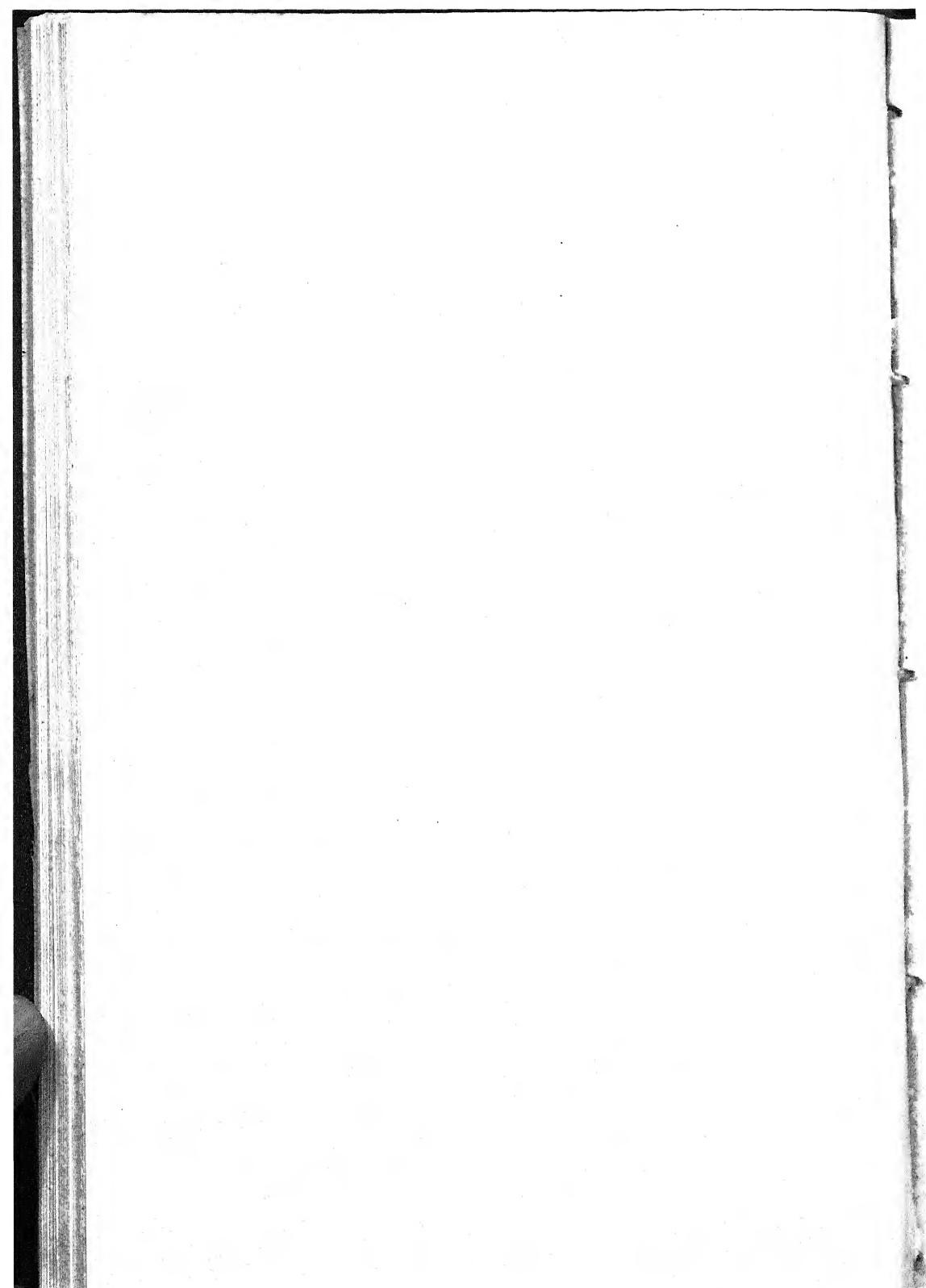
A SOLUBLE SUBSTANCE IN CORNSTALKS THAT RETARDS GROWTH OF DIPLODIA ZEAE IN CULTURE [1]

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INTRODUCTION

As a rule, corn (*Zea mays* L.) plants are resistant to stalk rot, caused by *Diplodia zeae* (Schw.) Lév., during the period of their vegetative growth. Increase in susceptibility occurs after pollination. According to Koehler and Holbert (*14*),[3] the disease seldom reaches the stage where it becomes noticeable until the ears are in the milk stage or the kernels have dented. Thus differences in susceptibility among lines and among individuals within a line become evident as the plants near maturity.

The amount of stalk rot fluctuates from year to year. Weather and soil conditions affect host and parasite individually and their interaction in the course of disease development. As a consequence, field tests to determine the relative resistance of various lines must extend over a period of years.

It is recognized that resistance to disease may depend primarily on the anatomy or the physiology of the host plant or on various combinations of the two factors. In the case of corn, an erect, mechanically strong stalk and a large, well-anchored root system are desirable anatomical features from an agronomic point of view and tend to be interpreted as indicative of disease resistance.

With these conditions in mind, the present work was undertaken to study a phase of physiological resistance to stalk rot. The investigation sought evidence of the presence of and seasonal change in a substance in the expressed juice and ether extracts of stalks of yellow dent corn, resistant or susceptible to *Diplodia zeae*, that would affect the growth of the fungus in culture. It was presumed that the results of the laboratory tests would be relative and that, based on field material, they might be expected to vary from season to season; but it was thought that over a period of years trends might become evident that would correlate in some measure with the resistance and susceptibility of plants in the field.

MATERIAL AND METHODS

The corn plants used were grown on the University farms at Madison, Wis. At the beginning of the study, the susceptible inbred Ill. Lan and the more resistant Ill. R4 were the contrasting lines selected.

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Later, another inbred, Wis. 6, was used as the comparative standard for susceptibility to stalk rot. Other inbred lines tested at various times were Ill. L,[4] Ill. 90, Ill. Hy, Wis. 23, Wis. 26, Wis. R3, Ind. B2, Ohio 10, and Ohio 56. The hybrids used were the single crosses Ill. 90×Ill. K, Ill. R4×Ill. Hy, Ill. R126×Ill. Lan, Wis. R3×Wis. 22, Wis. 23×Wis. 24, Wis. 23×Ind. WF9, and Ind. WF9×Wis. 22.

Periodic collections of stalk tissue were made. The number of plants in each sample ranged from 2 to 15 in different years. The stalks were stripped of their leaves immediately after collection, and comparable portions of the stalk tissue between crown and ear node were used as samples. Freshly chopped portions were dried at 60° C. in an oven equipped with a fan. The dried material was enclosed in tin cans and stored at room temperature until needed. Later this material was ground in a Wiley mill, to pass a 2-mm. sieve, and extracted with ether. The excess of ether was distilled off, and the volume of the extract was adjusted so that each cubic centimeter represented 2 gm. of air-dry stalk meal. The largest number of extracts prepared in any one season was 84, in 1938. Samples of plants collected from the field were not replicated, because of insufficient time and space for the preparation of such large numbers of extracts.

The influence of the ether extracts of stalk tissue on the growth of *Diplodia zeae* in culture was determined by adding 4 to 10 cc. of the extract to 100 cc. of hot synthetic [5] or potato agar containing 2 percent of dextrose or sucrose. After the agar had cooled somewhat, the bottles were well shaken and the contents of each was poured into four 9-cm. Petri dishes. By the time the agar had solidified, no odor of ether remained. The addition of corresponding quantities of pure ether to the control plates did not affect the size of the colonies of the fungus. One colony was grown on each plate. At first, various types of inoculum were used; later, a single drop of spore suspension placed in the center of the plate was adopted as a standard procedure. The diameter of the colonies was measured after the plates had been stored for 3 to 7 days under ordinary laboratory conditions.[6] Temperature, humidity, and light were not controlled in the room, nor was the inoculum used in the different platings of uniform age. Hence, colonies in one plating might differ in size from colonies of the same age plated at another time. However, in any one series, the conditions are thought to have been uniform and the colonies comparable. During the course of the work, several strains of *D. zeae* were used without material difference in the results obtained. The same was true of the agars used. Because the fungus grew more slowly on synthetic agar it was preferred when large numbers of colonies were to be measured.

Freshly chopped stalk tissue was ground for juice extraction in a Nixtamal mill. The juice was expressed in a Carver press under

[4] P. E. Hoppe has grouped a number of lines used in this work, with regard to their general resistance or susceptibility to stalk rot in the field, as shown by premature death of the plants or by the extent of discoloration visible on the surface of the stalks after hypodermic inoculation with *Diplodia zeae* in 1 year or more. His classification is as follows: Very susceptible, Ill. Lan and Wis. 6; susceptible, Ill. R4, Wis. 23, and Wis. 23×Wis. 24; intermediate, Ohio 10, Ill. L, Ill. 90, and Wis. 23×Ind. WF9; resistant, Wis. 26, Wis. R3, and Ohio 56; very resistant, Ind. B2 (one season), Wis. R3×Wis. 22, and Ind. WF9×Wis. 22.

[5] Asparagin, 2 gm.; KH_2PO_4, 2 gm.; $MgSO_4$, 1.25 gm.; carbohydrate, 20 gm.; agar, 17.5 gm.; distilled water, 1,000 cc.

[6] In the discussion of results obtained, unless otherwise indicated, the terms "resistance" and "susceptibility" as applied to the host will refer to the retarding effect of the juices and extracts on the growth of *Diplodia zeae* in culture.

pressures of 6,000 to 8,000 pounds, filtered through a Büchner filter, and made sterile by passage through a Seitz filter. A part of the filtrate was used in hanging drops to test the effect of the juices on the germination and growth of the spores of *Diplodia zeae*. Agar was added to another portion of the juice to make a 1.7-percent agar medium, which was autoclaved and used for plate study of the growth of the fungus. In 1937, juice agar was prepared by adding agar to expressed juice that had been centrifuged for 5 minutes instead of being filtered. This method was satisfactory and had the advantage of greater speed of preparation. Autoclaving at 15 pounds' pressure for 30 minutes, or at 20 pounds for 15 minutes, did not destroy the growth-retarding principle in the juice. Quadruplicate plates were used, and each plate contained approximately 25 cc. of medium.

When periodic collections of healthy stalks were made, a few similar plants in each plot were inoculated with a spore suspension of *Diplodia zeae* introduced into the stalk by means of a hypodermic needle. These stalks, which were intended to indicate the relative susceptibility to stalk rot, were harvested at intervals and split, and the lesions were photographed.

EXPERIMENTAL STUDIES

INFLUENCE OF SUBSTANCES OBTAINED FROM CORNSTALKS ON GROWTH OF FUNGI

Ether Extracts from Normal Stalks

The study of the influence of ether extracts of stalk tissue on the growth of *Diplodia zeae* in culture was begun in a preliminary way in 1934 and was continued from 1935 through 1940. In all, 245 extracts were prepared and added to agar, and approximately 4,700 colonies were measured in as many plates. Relative growth of the fungus on any series of extracts varied so little in repeated platings that averages of few or many colonies seldom altered materially the trends shown (table 1). The inbreds chosen for test were selected

TABLE 1.—*Effect of 4-percent ether extracts of various corn lines on growth of Diplodia zeae, 1935*

Line and harvest date	Mean diameter of colonies on quadruplicate plates		Mean diameter for 8 colonies	Colonies in all platings	
	4-day-old potato-sucrose agar cultures	7-day-old synthetic-sucrose agar cultures		Total	Mean diameter
	Cm.	*Cm.*	*Cm.*	*Number*	*Cm.*
Ill. Lan:					
Aug. 28	5. 6	5. 2	5. 4	36	5. 6
Sept. 25	6. 4	6. 4	6. 4	24	6. 8
Oct. 9	8. 4	8. 9	8. 7	36	8. 8
Ill. 90 × Ill. K:					
Aug. 28	6. 5	5. 8	6. 2	36	6. 4
Sept. 25	6. 8	7. 3	7. 1	20	7. 1
Oct. 18	8. 6	7. 9	8. 3	16	8. 8
Ill. R4:					
Sept. 5	4. 4	4. 2	4. 3	24	4. 1
Sept. 25	5. 2	5. 2	5. 2	28	4. 4
Oct. 18	3. 4	3. 4	3. 4	20	3. 3
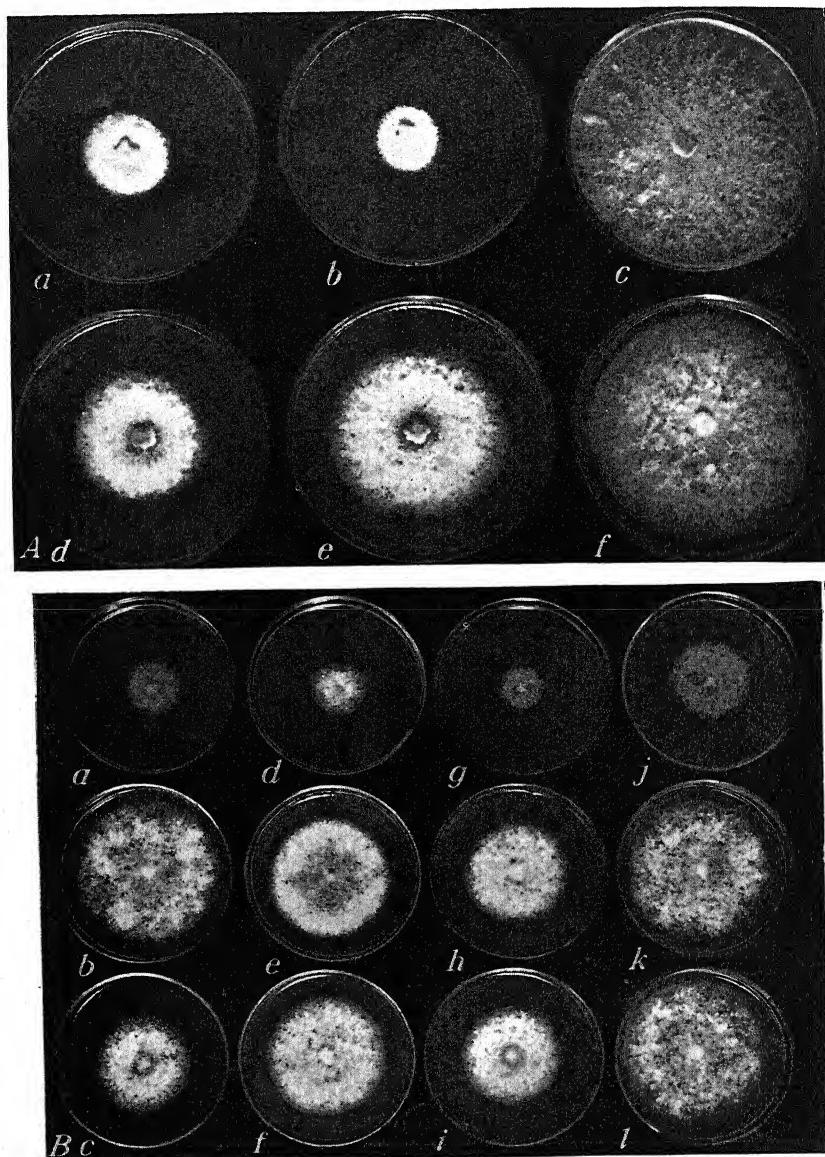
FIGURE 1.—Colonies of *Diplodia zeae* on (*A*) potato-dextrose agar and (*B*) synthetic agar, containing ether extracts from stalks of various inbreds collected on different dates. *A*, *a* and *b*, Extracts from inbred Ill. R4, collected September 5 and October 18, 1935, respectively; *c*, control (no extract added); *d–f*, extracts from inbred Ill. Lan, collected August 28, September 25, and October 9, 1935, respectively. *B*, Extracts from the following inbreds: *a–c*, Wis. 6; *d–f*, Wis. 26; *g–i*, Wis. 23; *j–l*, Wis. R3, each collected August 5 and 25 and September 9, 1938. respectively.

for a variety of characters. They showed a range in the number of days required to reach maturity, in the strength of their stalks and extent of their root systems, and in their resistance or susceptibility to stalk rot. Of the inbreds tested, only the 11 lines that had been used for 2 years or more or had entered into the hybrids have been included in this report together with 5 hybrids.

The results obtained indicate in general the presence in cornstalks, during the period of vegetative growth, of an ether-soluble substance that retards the spread of *Diplodia zeae* in culture (fig. 1). This substance tended to diminish for a time after pollination. Some lines consistently reached higher levels of susceptibility than other lines; some attained their maximum early, after which susceptibility, as
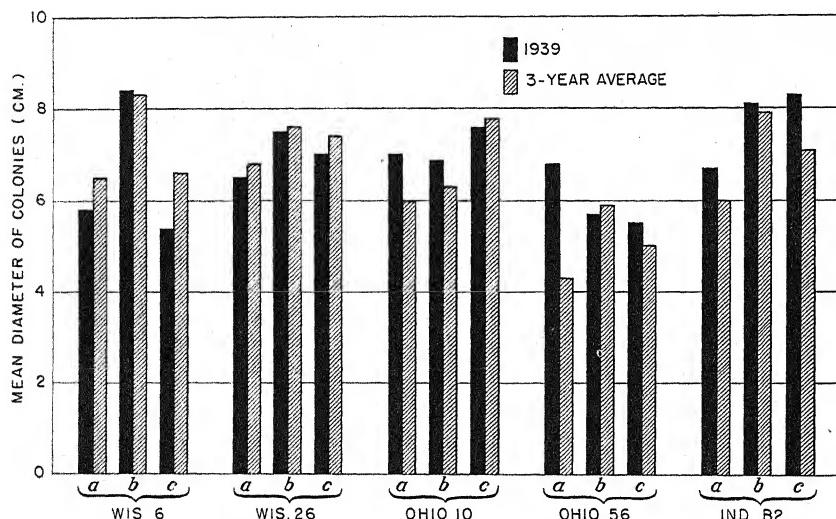
FIGURE 2.—Seasonal trends in 1939 and 3-year averages, in the growth-retarding substance of ether extracts from five inbred lines as shown by the mean diameters of colonies of *Diplodia zeae* on agar containing ether extracts of stalks harvested in 1939 on (*a*) August 1, (*b*) August 21, and (*c*) September 8 and in 1937 to 1939, inclusive, on approximately corresponding dates.

indicated by the size of the fungus colonies, declined more or less. In other lines, maximum susceptibility was delayed until late in the season (figs. 1 and 2; table 1). Moreover, trends in short-season inbreds and long-season inbreds were likely to differ somewhat over the same three-collection period. Other complicating factors to be considered were the possible variations among plants within lines and the small number of plants in the sample collections in some years.

Differences in the seasonal change shown depended to some extent on differences in the stage of development of the plants on the date of collection. This in turn was influenced from year to year by weather conditions (*21*) (table 2) during the growing season. In some years pollination began in late July; in other years it was delayed until mid-August. When plants were collected near the time of pollination, the earlier the collection was made the greater in general was the growth-retarding effect of the ether extracts. In some lines the

seasonal trend shown varied little from year to year (fig. 2, Wis. 6 and Wis. 26). In other lines, data obtained in different years appeared at first glance to conflict. Such was the case with the extracts of Ohio 56 over a 3-year period (fig. 3), until the stage of development of the plants at the time of collection was considered. Weather conditions in 1937 and 1939 (table 2) were such that the plants were well advanced at the time of the first collections. In 1938, the temperatures were lower, with greater rainfall, and the plants developed more slowly. The first collection of that year was made 9 days before pollination
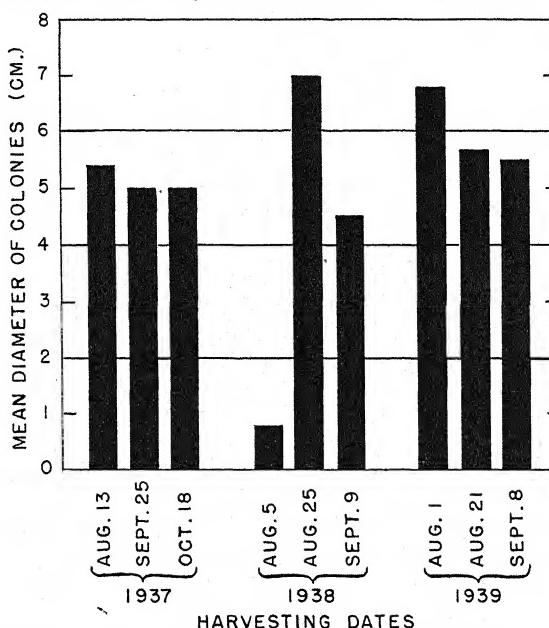
FIGURE 3.—Mean diameters of colonies of *Diplodia zeae* on agar containing ether extracts from stalks of inbred Ohio 56, collected on different dates.

began in Ohio 56. Thus the plants of the second collection of Ohio 56 in 1938 approximated in development those of the first collections in 1937 and 1939.

TABLE 2.—*Temperature and rainfall May to August, 1934–40, at Madison, Wis.*

| Year | Temperature (sum of average deviations, May to August) | Rainfall | | Year | Temperature (sum of average deviations, May to August) | Rainfall | |
		Total, May to August	Deviation from normal			Total, May to August	Deviation from normal
	°F.	Inches	Inches		°F.	Inches	Inches
1934	+16.8	9.22	−5.48	1938	+3.1	15.84	+1.14
1935	−3.3	14.74	+.04	1939	+11.6	8.22	−6.48
1936	+15.4	9.99	−4.71	1940	−.7	17.87	+3.17
1937	+9.0	6.80	−7.90				

The mean diameter of colonies of *Diplodia zeae*, on agars containing the ether extracts of 11 inbreds of diverse character, is shown graphically in figure 4 for each of 3 collections per season. The results

obtained during a 5-year period indicate that, on the average, maximum resistance occurred at the time of the first sample collection. Susceptibility increased to its maximum at the time of the second collection, and in this group of inbreds the mean continued at the same level through the third collection. In the case of the 5 single crosses, no differences in the general direction of their individual trends were shown during the period of sampling, although the pitch of the curves varied. In each hybrid line, as in the mean (fig. 4), maximum susceptibility was delayed until the time of the third collection.

The growth-retarding influence of the ether extracts of various inbreds was not specific for *Diplodia zeae*. The spread of colonies
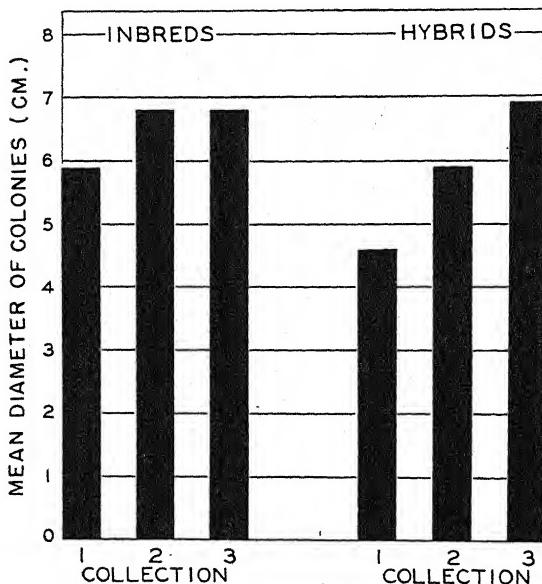
FIGURE 4.—Seasonal susceptibility of corn to stalk rot as indicated by the mean diameters of colonies of *Diplodia zeae* on agar containing ether extracts of stalks of plants collected on 3 dates each year. The means involve 11 inbred lines and 5 hybrids, differing among themselves in the number of days required to reach maturity and in degree of resistance shown in the field. Seven of the inbreds were grown for 3 successive years, between 1935 and 1939, inclusive; 1 hybrid was grown in 1935 and 4 in 1940.

of *Nigrospora sphaerica* (Sacc.) Mason (*Basisporium gallarum* Moll.) likewise was retarded more by the extract from stalks of Ill. R4 than by that from stalks of Ill. Lan (fig. 5, *A*).

The effect of the extracts on the growth of *Gibberella zeae* (Schw.) Petch (*G. saubinetii* Auct.; not *G. saubinetii* (Mont.) Sacc.) (fig. 5, *B*) also followed the general trends shown for *Diplodia zeae*. On the other hand, the spread of *G. fujikuroi* (Saw.) Wr. (*Fusarium moniliforme* Sheldon) appeared to be affected little by any of the extracts used in the agar upon which it was grown. The colonies were measurable earlier than the colonies of *Diplodia zeae*, and differences in size, occasioned by the dates of collection of the stalks or by
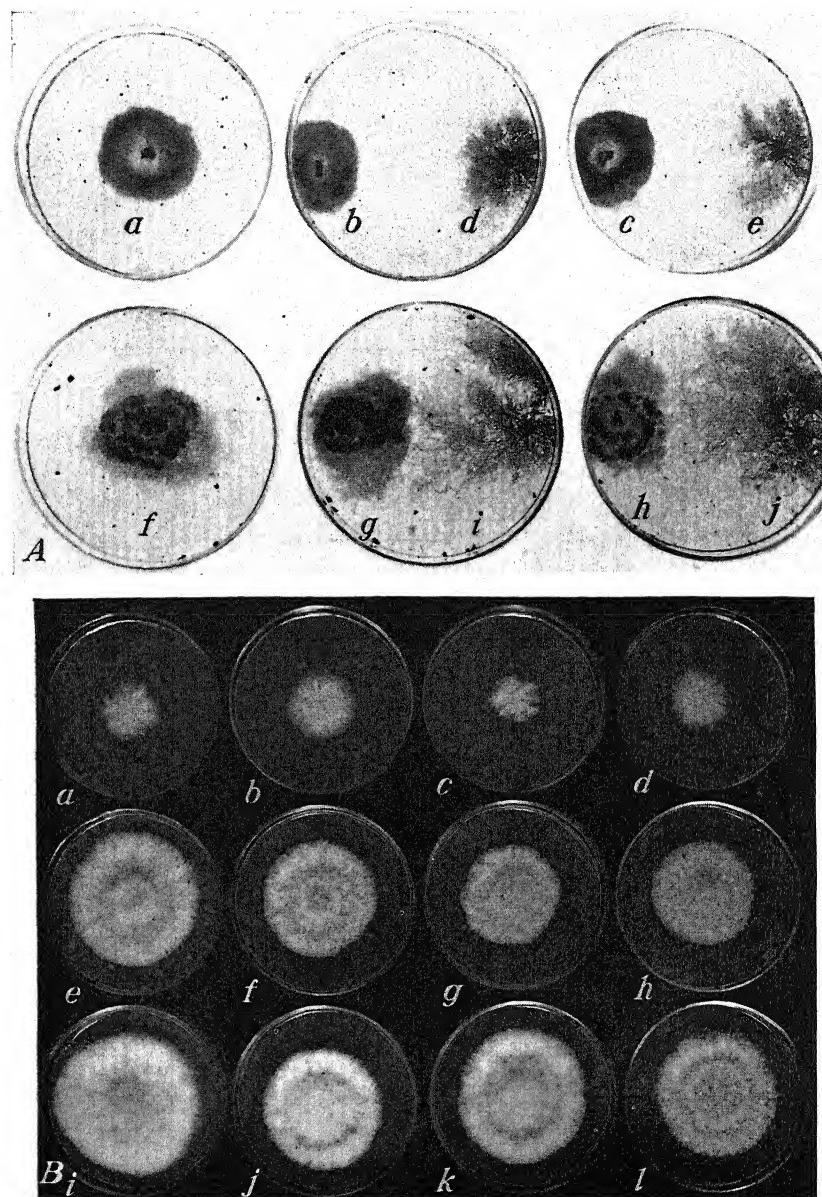
FIGURE 5.—Seven-day-old colonies of *Diplodia zeae*, *Nigrospora sphaerica*, and *Gibberella zeae* on synthetic agar containing 4 percent of ether extract of stalks of various inbreds. *A: a–c, D. zeae* on ether extract from inbred Ill. R4; *d* and *e, N. sphaerica; f–h, D. zeae* on ether extract from inbred Ill. Lan; *i* and *j, N. sphaerica.* *B, G. zeae* on ether extract from various inbreds: *a–d,* Ohio 56; *e,* Wis. R3; *f,* Wis. 26; *g,* Wis. 6; *h,* Wis. 23, collected August 5, 1938: *i,* Wis. R3; *j,* Wis. 26; *k,* Wis. 6; *l,* Wis. 23, collected September 21, 1938.

different strains of corn, were small. Sectoring in *G. fujikuroi* was increased by all the extracts.

WATER-SOLUBLE FRACTION OF ETHER EXTRACT

One test was made of the effect of a water-soluble fraction of the ether extract on the growth of *Diplodia zeae*. A 4-cc. portion of the ether extract of stalk tissues of Wis. 6 and Ohio 10, harvested on August 25, and of Ohio 56, harvested on September 9, 1937, was added to a 100-cc. portion of distilled water in each case; the mixture was well shaken, and the ether was evaporated off. The remaining filtered aqueous solutions were used instead of water in the preparation of synthetic agar (fig. 6, *e–g*). At the same time, synthetic agar prepared in the usual way received 4 percent of ether extract (fig. 6,
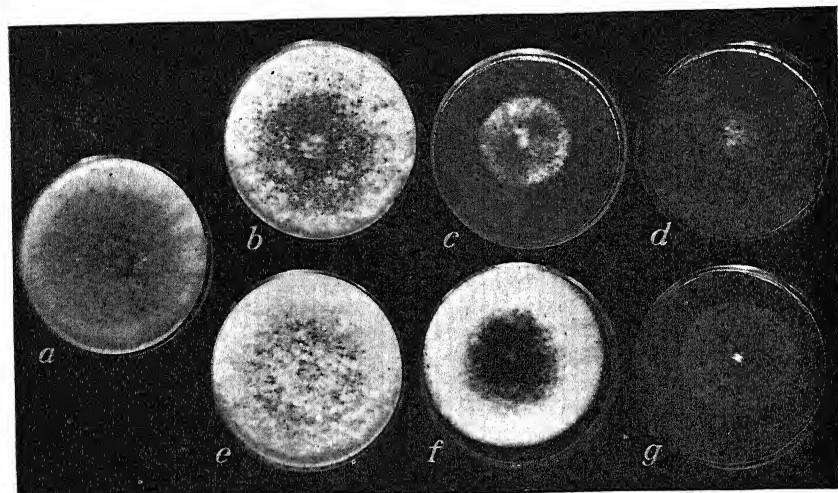
FIGURE 6.—Colonies of *Diplodia zeae* on synthetic agar containing ether extracts or water-soluble fractions of the extracts: *a*, Control (no extract added); *b–d*, ether extract of stalks of inbreds, Wis. 6 and Ohio 10, harvested August 25, 1937, and Ohio 56, harvested September 9, 1937, respectively; *e–g*, water-soluble fractions of the extracts from same inbreds.

b–d). Figure 6, *f* and *g*, indicates that a portion of the ether-soluble growth-retarding substance was soluble in water.

Attempted comparisons of the relative solubility in ether, alcohol, and water of the toxic substance in the cornstalk tissue were not successful, because of the large amounts of other soluble materials extracted from the stalk meal by both alcohol and water, which apparently served as nutrient material for the fungus.

ETHER EXTRACTS FROM STALKS OF LEAF-CLIPPED AND DEFRUITED PLANTS

In 1938 and 1939, attempts were made to determine whether the ether-soluble substance in the stalk tissue could be modified either by preventing kernel formation or by partial defoliation of the growing plant. In 1938, the ear shoots were bagged and, although fertilization did not occur, the cobs and husks grew to normal size. In 1939, the
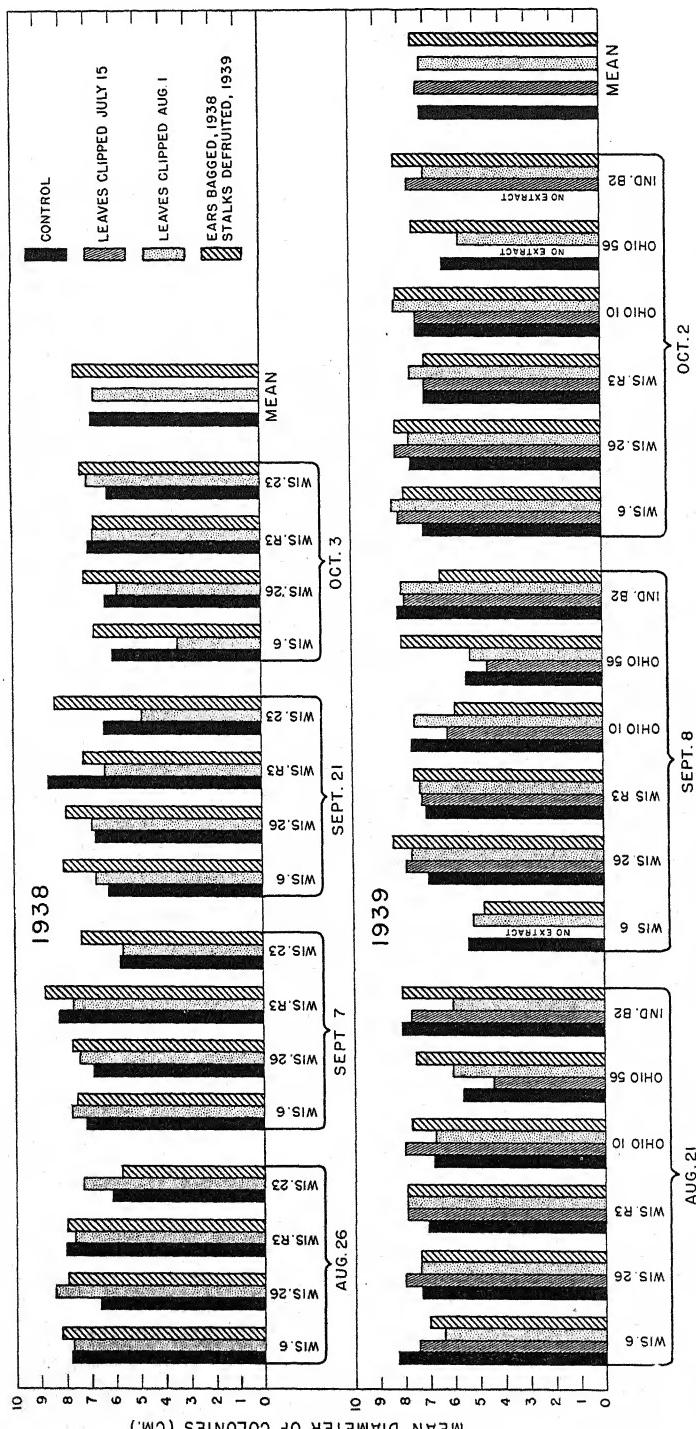
FIGURE 7.—Effect of partial defoliation and of the prevention of kernel formation on the growth-retarding substance of ether extracts of plants of various lines so treated in 1938 and 1939. Mean diameters of colonies of *Diplodia zeae* on synthetic agar containing ether extracts of inbreds Wis. 6, Wis. 26, Wis. R3, and Wis. 23, harvested on different dates in 1938; of inbreds Wis. 6, Wis. 26, Wis. R3, Ohio 10, Ohio 56, and Ind. B2, harvested on different dates in 1939; and the mean for each treatment in 1938 and in 1939.

ears were removed at pollination time. In that year the distal half of all leaves of other plants of each line were removed, some on July 15 and others on August 1. The leaves of the plants clipped on July 15 continued to elongate, so that the final result of that clipping was the removal of approximately only one-third of the normal leaf area.

Visible differences in the plants followed these treatments. On August 9, 1939, it was noted that leaf clipping appeared to have retarded normal ear development in the single-eared inbreds and to have reduced the number of ears in the two- and three-eared strains. Most of the plants of Wis. 6 and Ohio 56 whose leaves were clipped on July 15 were showing only one ear; those clipped on August 1, two ears, one of which was small; while the unclipped control plants had what promised to be two good ears in the process of development. The plants from which the ears had been removed were the most resistant to lodging; their stalks remained green longer and were harder to cut with a knife and to break than the stalks subjected to other treatments. The pith of the defruited plants of Wis. 6, and to a less degree those of Ohio 10 and Wis. R3, was mottled with brown throughout the portion of the stalk harvested. The discoloration of the cell walls and the plugging of the intercellular space were similar in appearance to the symptoms that accompany fungus invasion or wounds or that result from the overnutrition of certain cells (*11, pp. 189–190*). Platings and sections of this discolored tissue failed to reveal the presence of a fungus.

Freehand sections of defruited stalks collected in mid-September 1939, when treated with potassium iodide-iodine solution, showed the presence of considerably more starch around the bundles in the outer pith and subepidermal layers than was seen in the partly defoliated plants. In sections of stalks of Ohio 56 harvested on September 19, it was noted that starch extended farther toward the center of the stalk than in the other strains examined.

The effect of the treatments on the ether-soluble substance in the stalks of the individual lines, in 1938 and 1939, as measured by platings, is shown in figure 7. Other workers (*4, 5, 12*) have reported changes in resistance or susceptibility of plants in the field following similar treatments and have found that increased resistance accompanied changes resulting in an increase in the carbohydrate content of the stalks. In the present work there was no indication, in the erratic values obtained in both years (fig. 7), that prevention of kernel formation and the consequent increase in the carbohydrate content of the stalk consistently resulted in increased toxicity of the ether extract; nor was a uniform increase in susceptibility indicated in the stalk extracts of the partly defoliated plants as compared with those of the control plants.

EXPRESSED JUICE FROM NORMAL STALKS

Juice was expressed from stalks of Ill. R4 and Ill. Lan harvested on August 25, September 21, and October 8, 1936. The sap expressed from the plants of the first harvest was rendered sterile by passage through a Seitz filter. Spores of *Diplodia zeae* were germinated in hanging drops of the sterile juice. After 18 hours at room temperature, elongation of the germ tubes in the juice of Ill. R4 was approximately 25 percent less than that in the juice of Ill. Lan. In the stalks

harvested September 21 and October 8, when juice agar was used, a consistent difference between the two inbreds was shown by the size of the colonies. In comparable sets of plates in 1936, the diameters of all individual colonies, as well as their means, were smaller on the Ill. R4 juice agar than on the Ill. Lan juice agar (figs. 8 and 9, *A*).

Similar experiments with the fungus on juice agars of 7 inbreds were conducted in 1937. Weekly collections were made from August 13 to September 17. The method employed was that used in 1936, except that the expressed juice was centrifuged for 5 minutes instead of being filtered.

The juice from two to four plants of each inbred was used and platings were made after each collection (fig. 8). Line differences were shown in the weekly collections; and in 1937, as in 1936, colonies of
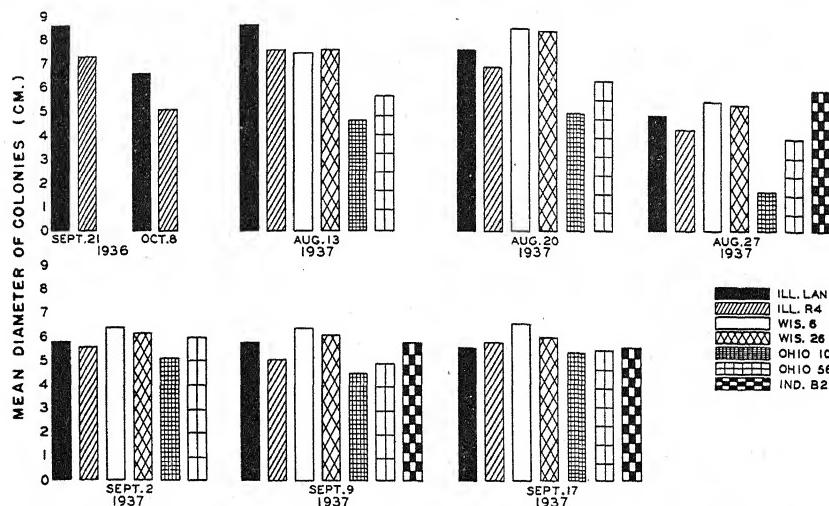
FIGURE 8.—Diameters of colonies of *Diplodia zeae* on agar containing juices of various inbreds. The sizes of the colonies may be compared for a single collection but not necessarily for different collection dates in 1937, for in that year platings were made immediately after each collection and no plating included all collections.

the fungus on the juice agars of Ill. R4 were, with one exception, smaller than those on the corresponding juice agars of Ill. Lan (fig. 8).

In order to determine if the effects of the various juices on the growth of *Diplodia zeae* could be ascribed to differences in pH, tests were made of the juices of plants collected on September 21, 1936, and on August 20, 1937. These tests gave no indication either of wide differences in the pH of the juices from the various lines or of a relation between the size of the colonies and the acidity of the medium at the time the plates were poured (table 3).

Because of an insufficient amount of juice in some samples no complete series of plates involving the juices of each line for all dates of collection was made. Hence, no comprehensive test was obtained of the seasonal change in the inhibiting action of the juices. Platings made with the juice agars of three inbreds harvested on August 13,

20, and 26 showed less retardation in the colony growth on the later dates than on August 13 (table 4).   Figure 9, *B*, likewise shows a seasonal change in the early-maturing Wis. 6 and the late-maturing Ohio 10.

Similarity in the strain differences of juices and the extracts of seven inbred lines is shown in figure 10, although the plants were sampled over different periods of time.   The relative susceptibility of Ill. Lan and Ill. R4 was the same for the juices as for the extracts.   On the juice
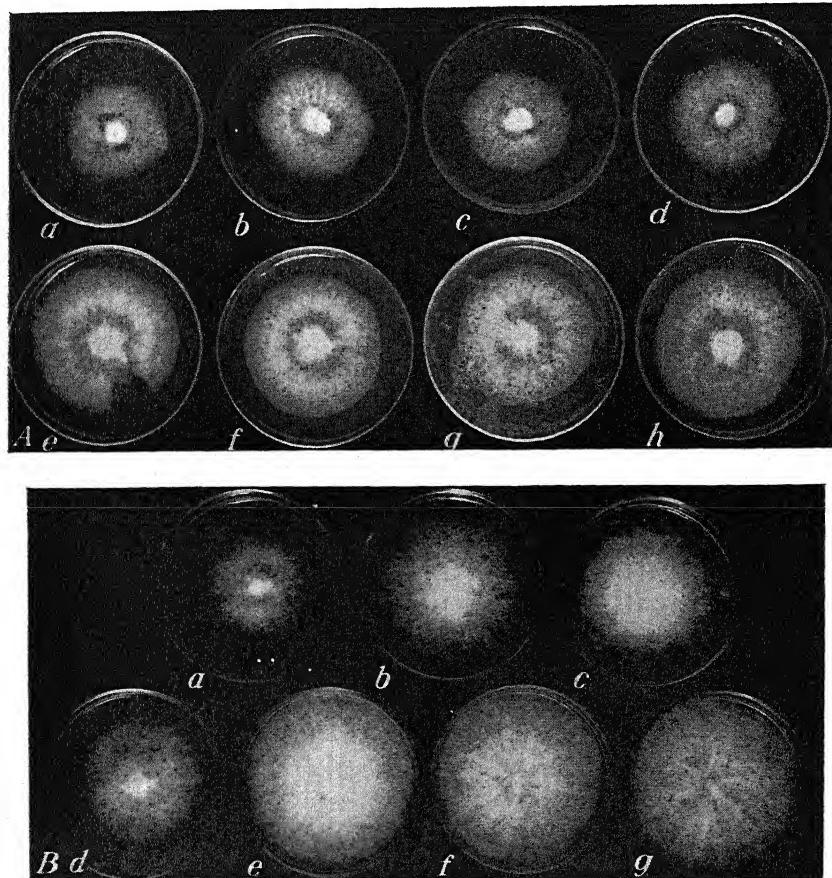
FIGURE 9.—Colonies of *Diplodia zeae* on juice agar: *A*, Colonies 3 days old on stalk juice from (*a–d*) inbred Ill. R4 and (*e–h*) inbred Ill. Lan, collected September 21, 1936; *B*, colonies 4 days old on stalk juice from (*a–c*) inbred Ohio 10, collected August 20 and September 2 and 17, 1937, respectively, and from (*d–g*) inbred Wis. 6, collected August 13 and 26 and September 2 and 17, 1937, respectively.

agars of six collections in 1937, the colonies on agar containing the juice of Ohio 10 were on the average the smallest.   On the ether extracts of plants from three collections each, in 1937, 1938, and 1939, the colonies on the extracts of Ohio 56 were the smallest and those of Ohio 10 relatively large.   Considering both media, the two Wisconsin inbreds gave the largest colonies and Ohio 56 the smallest.

TABLE 3.—*Diameters of colonies of Diplodia zeae grown on juice agars of plants collected September 21, 1936, and August 20, 1937, and the pH of the respective agars*

Inbred	Diameter of colonies		pH of juice agar	
	Sept. 21, 1936	Aug. 20, 1937	Sept. 21, 1936	Aug. 20, 1937
	Cm.	*Cm.*		
Ill. Lan	8.3	6.4	5.20	5.55
Ill. R4	6.7	5.6	5.27	5.46
Wis. 6		7.4		5.48
Wis. 26		7.4		5.84
Ohio 10		4.1		5.88
Ohio 56		5.4		5.69

TABLE 4.—*Trends in susceptibility in three inbred lines as indicated by the diameters of Diplodia colonies grown on juice agars of stalks harvested on different dates in 1937*

Inbred line	Diameter of colonies		
	August 13	August 20	August 26
	Cm.	*Cm.*	*Cm.*
Wis. 6	3.6		6.6
Ill. R4	4.6		4.7
Ohio 56	3.4	4.2	
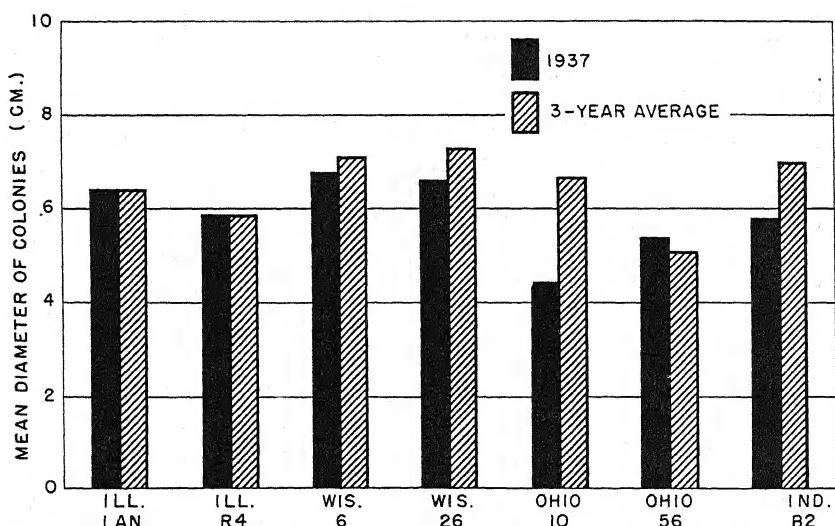
FIGURE 10.—Strain differences in resistance or susceptibility to stalk rot in various inbreds, as shown by the mean diameters of colonies of *Diplodia zeae* on juice agars of these inbreds, from plants collected at weekly intervals from August 13 to September 17, 1937, and on ether-extract agars of the same inbreds, in 3-year averages, from plants collected as follows: In 1935, August 28, September 25, and October 18; in 1936, August 20, September 15, and October 6; in 1937, August 13 and 27 and September 9; in 1938, August 5 and 25 and September 9; and in 1939, August 1 and 21 and September 8. The 3-year averages for Ill. Lan and Ill. R4 are for the years 1935 to 1937, inclusive; the others, for 1937 to 1939, inclusive.

These data appear to permit the conclusion that there is little difference in physiological susceptibility between Wis. 6 and Wis. 26. In the field, the expression of this similarity is modified by differences in the anatomical strength of the stalks and the extent of root development. Wis. 6 has a weak stalk likely to break or lodge; the stalk of Wis. 26 is strong and tends to remain erect.

SPREAD AND CHARACTER OF ARTIFICIALLY INDUCED STALK ROT

Individual lines and plants varied in their reactions to hypodermic inoculation with spore suspensions of *Diplodia zeae*, but generally resistance appeared to be relatively high until the time of pollination or shortly thereafter. Lesions resulting from inoculations made in
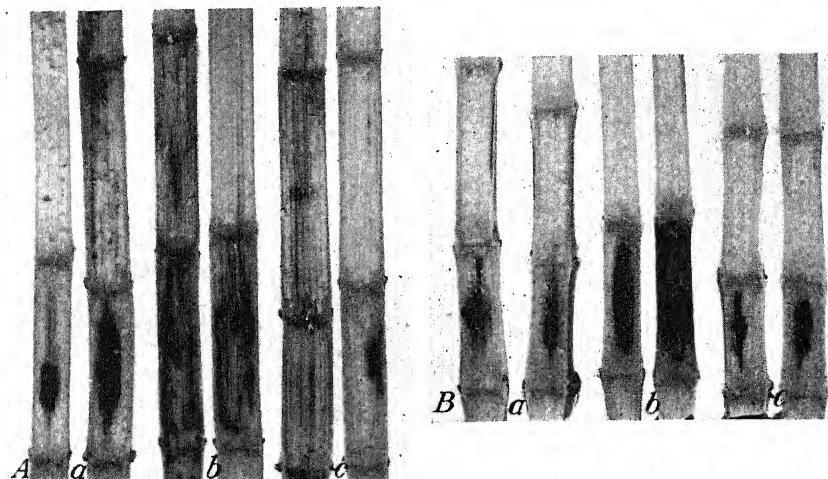
FIGURE 11.—Spread and character of lesions in two stalks of each collection of inbreds: (A) Wis. 6 and (B) Ohio 56, resulting from inoculation with *Diplodia zeae* on (a) August 15, (b) August 24, and (c) September 9, 1938. Stalks collected September 22, 1938.

late July or early August were smaller than those resulting from inoculations made in late August or early September, despite the advantage of the former in length of time for development before the stalks were harvested (figs. 11 and 12). Lesions resulting from the early inoculations were darker in color than those resulting from late inoculations; those resulting from the later inoculations in the more resistant strains also were darker than those in the susceptible lines inoculated on the same date. In the hybrids inoculated, maximum susceptibility generally occurred later than in the inbreds (figs. 4 and 12).

Sections through the dark lesion in the stalk of Ohio 56 (fig. 13, A) show a comparatively small portion of the darkened area occupied by the fungus. The discoloration of the cell walls and the plugging of the intercellular spaces extend well in advance of the hyphae (fig. 13, B and C). A lighter colored lesion in a stalk of the susceptible inbred Ill. Lan is shown in figure 14, A. Less retardation in the spread of the fungus and less change in the tissue of the host (fig. 14, B and C)

are indicated in this stalk. The differences shown in figures 13 and 14 were in stalks inoculated on the same date and harvested at the same time.
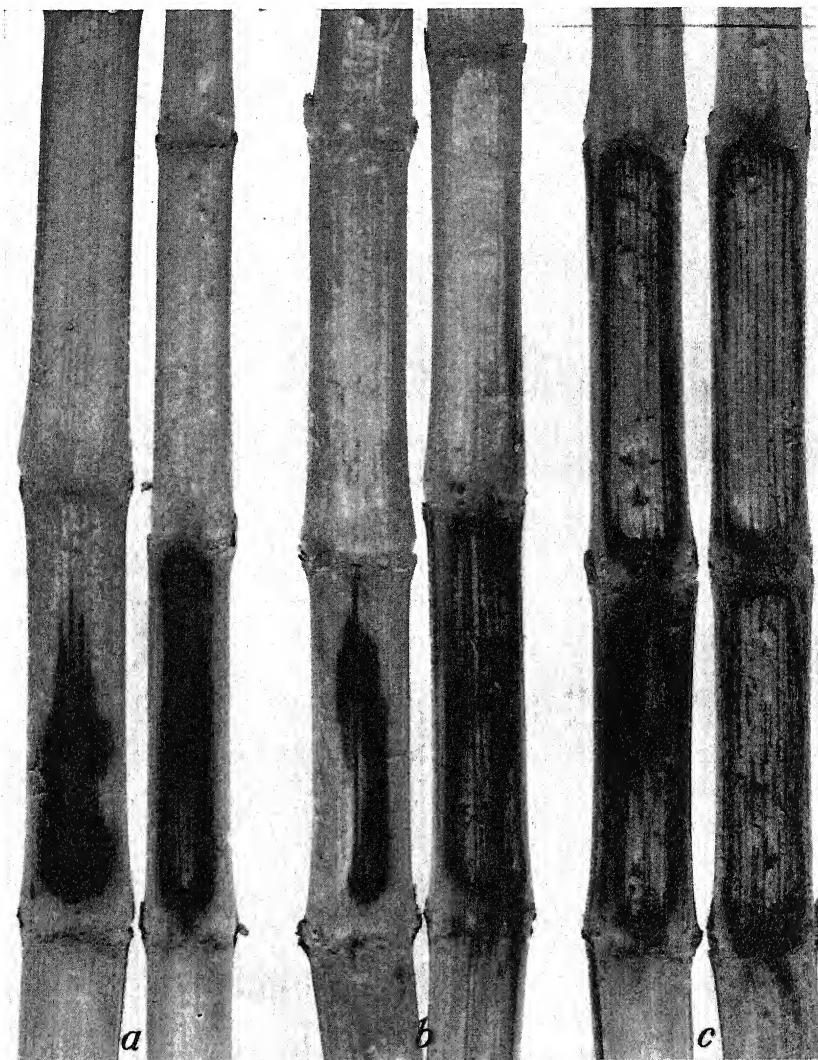
FIGURE 12.—Spread and character of lesions in stalks of hybrid Ind. WF9 × Wis. 22, resulting from inoculation with *Diplodia zeae* on (*a*) July 29, (*b*) August 20, and (*c*) September 4, 1940. Stalks collected October 10, 1940.

DISCUSSION

The literature on plant diseases contains many references to disease resistance, but only a few of the later papers need be mentioned here in order to indicate some of the phases of the problems that have been investigated and the conclusions that have been drawn.
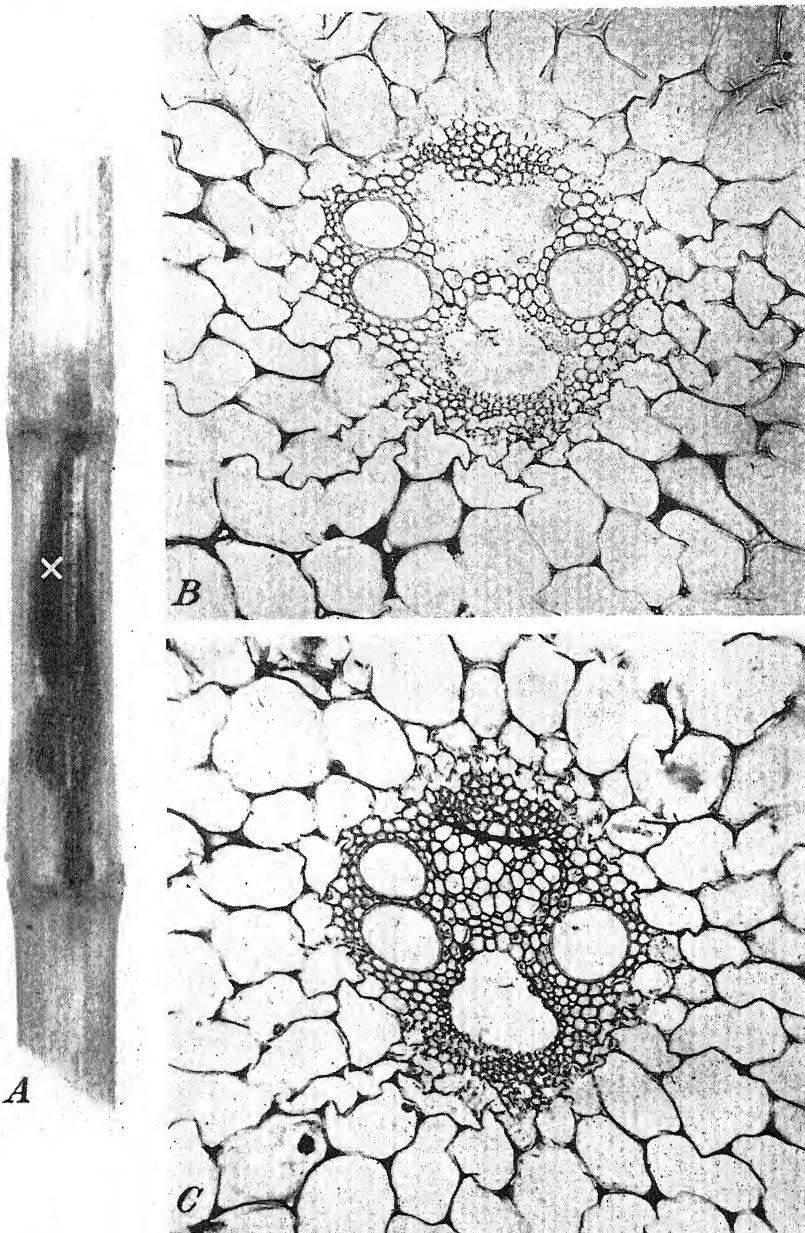
FIGURE 13.—*A*, Lesion in a stalk of inbred Ohio 56, inoculated with *Diplodia zeae* August 20 and harvested September 3, 1937. *B*, Section from location marked (x) in the dark-colored tissue in *A*, midway between puncture and node, showing plugging of intercellular spaces and discoloration of cell walls; section from fixed and embedded material, unstained, mounted in balsam. *C*, The same bundle on the next slide of the same series as section shown in *B*, stained with Flemming's triple stain to show the absence of fungus in this area. *B* and *C*, × 110.
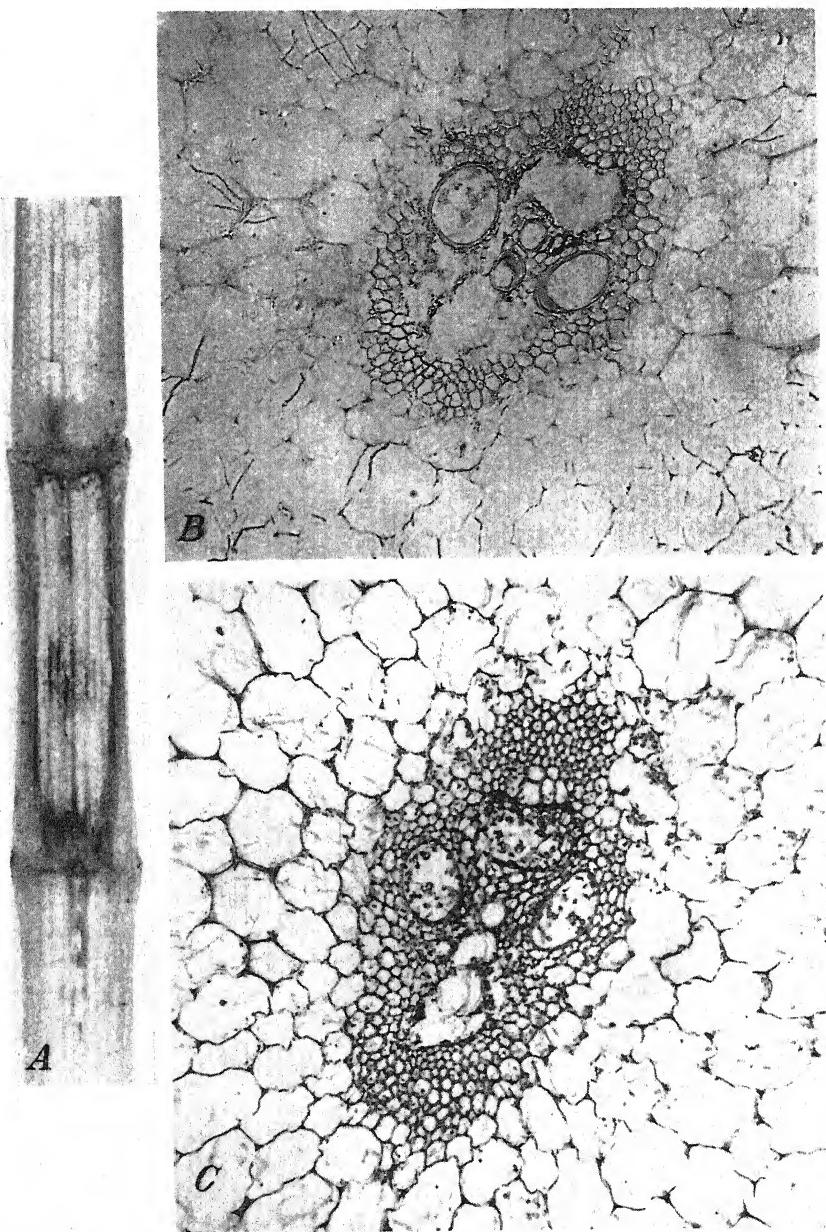
FIGURE 14.—*A*, Lesion in a stalk of inbred Ill. Lan, inoculated with *Diplodia zeae* August 20 and harvested September 3, 1937. *B*, Portion of a section from the light-colored area of the infected internode shown in *A*; little plugging of the intercellular spaces or discoloration of the cell walls has occurred; section from fixed and embedded material, unstained, mounted in balsam. *C*, Portion of another serial section of the same area as shown in *B*, stained with Flemming's triple stain to show hyphae permeating the cells of both pith and bundle. *B* and *C*, × 110.

With regard to the extent that stalk breaking indicates stalk rot, Copper et al. (*3, p. 177*) stated: "Data taken this year [1939] showed a very close relationship in some crosses and very little relationship in others."

Koehler [7] and Holbert et al. (*12*), from their observations of stalk rot in the field, agree that corn plants generally become increasingly susceptible as the ear approaches maturity.

Elliott (*7, p. 262*) found that "A change in the resistance of dent-corn plants to bacterial wilt apparently takes place in late July and August, after the plants have tasselled" and that "On dent corn, local leaf-blight lesions on maturing plants do the greatest damage."

Hart (*10*), Ivanoff and Riker (*13*), and Ranker (*18*) concluded that more than one type of resistance may have been present in the plants they studied. Smith and Walker (*20*) ascribed resistance to yellows to antagonistic chemical substances or physiolgical qualities in the roots of the cabbage plants. Other workers have linked resistance to the presence in host tissue of phenolic compounds (*6, 17*), protocatechuic acid (*1*), tannin (*2*), berberine (*9*), and catechol (*15*), substances known to be toxic to fungi. Moore (*16*) found water-soluble, growth-inhibiting substances in roots resistant to phymatotrichum root rot. Ezekiel and Fudge (*8, p. 786*), working with the same disease, stated: "The general immunity of monocots to root rot is due at least in part to the presence in the roots . . . of minute quantities of acidic, ether-soluble substances, possibly organic acids or esters." Reynolds (*19*), after experimenting with water extracts of different parts of flax plants, suggested that the quantitative results obtained may be correlated with the actively functioning protoplasm in the different parts.

Conclusions reached in the present study have been in substantial agreement with those in several of the articles cited. The juices and ether extracts of stalks harvested before or shortly after pollination were found to retard the growth of *Diplodia zeae* in culture to a greater degree generally than those of the later harvests. This accords with the results of the writers' inoculations in the field and with the occurrence of stalk rot as observed by Koehler and Holbert (*14*) and Holbert et al. (*12*).

Results were not altogether uniform from year to year, nor was there uniformity in the weather conditions under which the plants developed. In comparing results, therefore, one must remember that plants were not necessarily at similar stages of development on the same calendar dates in different years, nor were all lines at the same stage of maturity at any one time of collection. Despite these limiting circumstances, certain lines held their position of relative resistance or susceptibility with regard to the one factor of the resistance complex under consideration. During the years 1937 to 1939, in comparisons of several inbreds, the extracts of Ohio 56 showed on the average the smallest colonies of *Diplodia zeae* in each year. It may be noted here also that, under field conditions in three locations in Illinois in 1939, this inbred was fifth in a group of 48 inbreds, ranked according to their resistance to damage from diplodia stalk rot as determined in single crosses. In the test plot in southern

Illinois, it held first place; in northern Illinois near De Kalb, it was tenth in the list.[8]

Immunity from stalk rot was not found in any of the lines used for study; nor does it appear that the degree of resistance observed in strains in the field should be ascribed wholly to substances within the tissue that tend to retard fungus growth, or to the anatomical strength of the stalks. Both factors contribute to the well-being of the plant and are combined in different proportions in various lines. The seasonal change in resistance seems to be largely of a physiological nature. Differences between lines in field performance are influenced by both physiological and morphological factors. Advantageous as a good root system and a strong stalk are to the corn plant, they do not of themselves appear to insure resistance to or immunity from stalk rot, or to be combined necessarily with high physiological resistance. Ind. B2 has a stiff stalk and a good root system, but it has been described as susceptible to ear and stalk rots. On the other hand, Ohio 56 [9] is listed as resistant to stalk rot, but "stalk may break in late September."

After pollination there was a period in the life of each plant when the growth-retarding substance in the stalk reached its lowest level. This took place soon after pollination in some lines; in others it was delayed until late in the season. Consequently the pitch, as well as the height, of the curve of increasing susceptibility differed in individual lines during the period of sampling, and in some instances it had changed in direction before the third collection. It is not known whether this increase in the toxicity of the extract late in the season was due to the presence of the substance found in the stalks before pollination, for the chemical nature of these materials has not been determined. It seems probable, however, that changes in direction were not so much an indication of radical differences in the trend of the curve of susceptibility as of differences in timing and in the speed with which various lines passed through one or more phases of their development.

SUMMARY

Data are given for 11 inbred lines and 5 single crosses of corn (*Zea mays*) investigated from 1935 to 1940 for the presence in the stalks of an ether- or sap-soluble substance that retards the growth of *Diplodia zeae* in culture and for seasonal changes in the growth-retarding substance. The ether extracts of inbreds and hybrids collected before or shortly after pollination generally retarded growth of the fungus to a greater degree than those of later collections. Certain lines retained more of the growth-retarding substance throughout the maturation of the plant than did others, indicating a physiological type of resistance not necessarily associated with strength of stalk or morphological resistance.

Extracts that were effective in retarding the spread of *Diplodia zeae* also retarded the growth of *Gibberella zeae* and *Nigrospora sphaerica* but had little influence on the spread of *G. fujikuroi*.

The growth-retarding substance in the extract was stable to heat and soluble to some extent in water.

[8] KOEHLER, B., BOLIN, O., and COPPER, R. R. CORN INBREDS RANKED ACCORDING TO THEIR RESISTANCE TO DAMAGE FROM DIPLODIA STALK ROT AS DETERMINED IN SINGLE CROSSES IN 1939. Ill. Agr. Expt. Sta. Dept. Agron., 3 pp. 1940. [Processed.]
[9] [JENKINS, M. T., reporter.] REPORT OF THE FIRST CORN IMPROVEMENT CONFERENCE, HELD AT CHICAGO, ILLINOIS, DECEMBER 3, 1937. 40 pp. Washington, D. C. 1938. [Processed.] (See pp. 13, 24, 31.)

Removal of the ears did not consistently increase, nor did clipping the leaves decrease, the growth-retarding effect of the ether extracts of the plants so treated.

Expressed juice of the stalks contained a growth-retarding substance that showed seasonal change and differed in the lines tested much as did the ether extracts.

Results obtained by inoculating various inbreds and hybrids with *Diplodia zeae* also indicated that resistance was relatively high in all lines until the time of pollination and for shorter or longer periods in different lines thereafter.   The spread of the fungus was restricted in the dark lesions of resistant plants and was impeded but little in the lighter colored lesions of susceptible tissue.

The chemical nature of the growth-retarding substance is unknown, but the suggestion is made that differences shown in the physiological resistance of the various lines may be due not so much to radical differences in the general trend of the curve of susceptibility as to differences in timing and in the speed with which lines pass through the various phases of their development.

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DIFFERENCES IN THE COMPOSITION OF THE FRUITS OF CUCURBITA VARIETIES AT DIFFERENT AGES IN RELATION TO CULINARY USE [1]

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INTRODUCTION

According to Safford (*10*) [2] pumpkin and squash were used as food by the prehistoric inhabitants of the Americas. At present they are widely grown for canning, for the city market, for home consumption, and for stock feed. The cultural methods employed in their production as crop plants have been described by Jones and Rosa (*9*) and by Thompson (*14*). The fruits are often stored for winter use, and considerable study has been given to methods of storage and to the chemical changes that occur in the fruits during the storage period (*6, 8*). The importance of the crop for canning has stimulated considerable work on the composition of the fruits of different varieties (*6, 7, 11, 13, 16*) and on the changes in composition that take place during growth and development (*8*). Their composition as food products has been summarized by Chatfield and Adams (*4*) and Winton and Winton (*15*).

A study of the published reports upon the chemical composition of pumpkins makes it apparent that, in addition to variations attributable to climatic and soil conditions, there are consistent inherent differences in composition among varieties and also significant differences between the various stages of maturity in any given variety. For the most part, previous investigations have been concerned with small groups of varieties, and the studies have not been continued over the entire life period of the fruits.

The purpose of the present study was to determine how the composition of a large number of *Cucurbita* varieties differed at different ages from the time of bloom to the end of their storage life. Numerous cooking and canning tests were made in order to ascertain how the changes in composition affected their culinary use. To reduce the error arising from variations in seasonal conditions the work was continued over a period of 4 years.

MATERIALS AND METHODS

CULTURAL METHODS AND VARIETIES

The varieties for these studies were grown on a sandy loam of good fertility at the Arlington Experiment Farm, Arlington, Va. They were grown in rows 6.6 feet apart with plants approximately feet apart in the row. The seeds of all varieties except three were

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[1] Received for publication August 18, 1943.
[2] Italic numbers in parentheses refer to Literature Cited, p. 136.

obtained from American seedsmen; Charles Naudin, Iron Bark, and Faan Kwa were of foreign origin. The planting in each of the 4 years was made on June 10. This permitted the early vegetative development of the plants to occur during the warmer part of the summer. The young plants were periodically dusted with insecticides. Shallow cultivation to destroy weeds was continued as long as the plants were small enough not to be injured by it. The vegetative growth of most varieties completely covered the ground by late July or early August.

Two varieties were grown the first year, an additional 12 the second year, 13 more the third, and 9 more the fourth, making in all 36 varieties. Of these, 8 belonged to the species *Cucurbita pepo* L., 6 to *C. moschata* Poir., and 22 to *C. maxima* Duchesne (table 1).

Descriptions of all but a few of the varieties have been given by Castetter and Erwin (*3*). The fruits of the varieties of each group varied in shape, smoothness, and color of the surface, in color and texture of the flesh, and in many other characteristics. No varieties of the summer squash type were used, but both pumpkins and squashes of the so-called winter squash types were included.

TABLE 1.—*Varieties used of each species and number of years each was grown*

Species and variety	Years grown	Species and variety	Years grown
Cucurbita pepo:	*Number*	*C. maxima*—Continued.	*Number*
Connecticut Field	3	Golden Hubbard	4
Fordhook	2	Improved Hubbard (Green Hubbard)	3
Fort Berthold	2	Iron Bark	1
Golden Oblong	3	King of the Mammoths	3
New England Pie	3	Kitchenette (Baby Hubbard)	1
Omaha	3	Mammoth Chili	3
Table Queen	3	Mammoth Whale	2
Winter Luxury	3	Marblehead	1
C. maxima:		Sibley	1
Arikara	3	Warren	2
Banana	2	Winnebago	2
Blue Hubbard	2	*C. moschata:*	
Boston Marrow	2	Faan Kwa	1
Charles Naudin	1	Green Striped Cushaw	2
Chicago Warted Hubbard	1	Japanese Pie	2
Delicious	1	Large Cheese	4
Essex Hybrid	2	Mammoth Golden Cushaw	3
French Turban (Acorn)	1	Tennessee Sweet Potato	2
Gilmore	2		
Golden Delicious	3		

ANALYTICAL PROCEDURE

To obtain comparable material for the analyses, blossoms were tagged at the time of opening. The plants generally began flowering and setting fruit in August; the field was gone over at intervals of 1 or 2 days, and tags showing the date of blooming were attached to the young fruits. Samples were usually collected on the date of blooming and 10, 20, 30, 40, and 60 days later, thus making a series of 6 samples for each variety in each year in which it was grown. The total number of series of samples was 79. The 60-day samples in nearly all cases were taken in October. The crop was then harvested and placed in common storage at 40° to 50° F.

The sampling was continued on the stored material at 90, 120, and 165 days from date of bloom. The last samples were taken the latter part of February, when many of the fruits appeared to be nearing the end of their storage life. In 6 of the 79 series, sampling was discontinued for one reason or another before the end of the storage period.

At date of flowering 15 to 30 entire fruits were used in the samples; at all other sampling periods only the wall, or edible portion of the fruit, was included. For each of these samples fractional parts of 3 to 6 fruits were used. The material was chopped into small pieces, which were thoroughly mixed. Duplicate 100-gm. samples were then weighed and placed in glass jars; sufficient 95-percent alcohol was added to make the final concentration 75 to 80 percent. The alcohol was then heated to boiling, the boiling was continued for 2 or 3 minutes, after which the lids were clamped down upon the jars, sealing them and excluding most of the air until the analytical work was begun.

The samples were prepared for analysis by extracting the material with alcohol in order to separate the sugars, acids, tannins, and other soluble material from the acid-hydrolyzable polysaccharides, pectin, and fiber. The residue was dried, weighed, and used for the determination of the acid-hydrolyzable polysaccharides. A separate sample of the fresh material was dried for the determination of total nitrogen.

The alcohol-soluble solids were determined by taking triplicate 25-ml. aliquots of the alcoholic extract, evaporating the alcohol at 60° C. on a water bath, completing the drying in a vacuum oven over calcium oxide at 75° under a negative pressure of 28 inches of mercury, and weighing.

The methods used in the determination of the various constituents were those given by the Association of Official Agricultural Chemists (*2*). The volumetric permanganate method was used for sugars, the Loewenthal-Procter method for astringent materials, titration with 0.1 N sodium hydroxide for acids, the ferrous chloride method for nitrates, and the Gunning-Arnold method as modified to include nitrates for the total nitrogen.

EXPERIMENTAL RESULTS

EFFECT OF DIFFERENT FACTORS ON THE COMPOSITION OF CUCURBITA FRUITS

An examination of the detailed analytical results showed rather large differences in the extent to which many of the constituents of the fruits varied. To determine the significance of these variations analyses of variance were made for the 36 varieties grown 1 year, for the 27 grown 2 years, and for the 14 grown 3 years; these results are not entirely independent because the 1-year results included the varieties grown for 2 and 3 years and the 2-year results included the varieties grown for 3 years.

The data used in the analysis of variance are those obtained in the samplings up to and including those at 60 days from date of blooming, at which stage the fruits of all varieties were mature. The series of samples obtained during storage were incomplete in a few instances, and the omission of the storage data makes the results strictly comparable for all varieties. The grouping of the varieties according to the number of years in which they were grown greatly simplified the computations.

In addition to the mean squares, the coefficients of variability were calculated for use in comparing the variability of the constituents. The results for the fresh weight of the fruit and for each of the chemical constituents for the three groups of data are given in table 2.

TABLE 2.—*Results of statistical analysis of the data from 36 Cucurbita varieties grown for 1, 2, or 3 years and harvested at different stages of maturity*

Item and source of variation	3-year results [1]			2-year results [1]			1-year results [1]		
	Degrees of freedom	Mean square	Coefficient of variability	Degrees of freedom	Mean square	Coefficient of variability	Degrees of freedom	Mean square	Coefficient of variability
Total solids:									
Variety	13	155.756**	29.62	26	81.837**	25.47	35	36.831**	24.85
Year	2	17.017**	3.84	1	66.196**	4.49			
Age	5	176.045**	19.53	5	257.090**	19.80	5	190.957**	21.39
Variety × year	26	3.489	6.27	26	2.258	4.23			
Variety × age	65	12.329**	18.63	130	6.448**	15.99	175	4.838	20.14
Year × age	10	3.611	3.96	5	2.651	2.01			
Error	130	2.867	12.71	130	2.263	9.47	175		
Soluble solids:									
Variety	13	7.040**	12.40	26	4.699**	12.33	35	1.926**	11.54
Year	2	3.959**	3.65	1	8.365**	3.23			
Age	5	28.826**	15.56	5	44.750**	16.69	5	28.154**	16.68
Variety × year	26	.631**	5.25	26	.420**	3.69			
Variety × age	65	1.351**	12.15	130	.800**	11.42	175	.428	12.17
Year × age	10	.719**	3.47	5	.465*	1.70			
Error	130	.230	7.09	130	.196	5.63	175		
Total sugar:									
Variety	13	4.532**	15.02	26	3.071**	14.91	35	1.100**	13.41
Year	2	4.963**	6.16	1	8.828**	4.96			
Age	5	37.642**	26.84	5	61.382**	29.23	5	34.212**	28.16
Variety × year	26	.511**	7.13	26	.383*	5.45			
Variety × age	65	.949**	15.36	130	.581**	14.50	175	.313	15.93
Year × age	10	.878**	5.80	5	1.057*	3.97			
Error	130	.221	10.48	130	.222	8.97	175		
Reducing sugar:									
Variety	13	1.354**	10.61	26	.768**	9.59	35	.483	10.93
Year	2	.387	2.22	1	1.690**	2.79			
Age	5	11.956**	19.56	5	19.536**	21.20	5	14.989**	23.01
Variety × year	26	.191	5.63	26	.212	5.03			
Variety × age	65	.535**	14.92	130	1.202**	26.81	175	.277	18.51
Year × age	10	.219	3.74	5	.326	2.74			
Error	130	.155	11.36	130	.147	9.38	175		
Acidity:									
Variety	13	.01229**	15.54	26	.08468**	16.19	35	.00474**	16.20
Year	2	.00681**	4.54	1	.03080**	6.06			
Age	5	.08690**	25.63	5	.12388**	27.16	5	.07797**	24.84
Variety × year	26	.00092	6.01	26	.00101	5.59			
Variety × age	65	.00169**	12.88	130	.00133*	14.34	175	.00139	19.59
Year × age	10	.00206**	5.58	5	.00584**	5.90			
Error	130	.00100	14.05	130	.00094	12.05	175		

Source of variation	d.f.	Mean square	F	d.f.	Mean square	F	d.f.	Mean square	F
Total astringency:									
Variety	13	.000185**	12.36	26	.000134**	10.94	35	.000052**	10.77
Year	2	.000057	2.67	1	.000011**	1.95	5	.001501**	21.85
Age	5	.002796**	29.80	5	.004416**	27.51			
Variety × year	26	.000050*	8.00	26	.000046	6.41			
Variety × age	65	.000052**	12.89	130	.000037	12.80			
Year × age	10	.000140**	8.30	5	.000468**	8.96			
Error	130	.000031	14.06	130	.000032	12.00	175	.000028	17.61
Nitrate nitrogen:									
Variety	13	.000219**	19.43	26	.000117**	25.70	35	.000100**	23.08
Year	2	.002572**	26.12	1	.008967**	44.21	5	.000613**	21.64
Age	5	.000174**	10.75	5	.000155**	13.00			
Variety × year	26	.000036*	11.17	26	.000027	12.35			
Variety × age	65	.000035*	17.39	130	.000034	30.82			
Year × age	10	.000049*	8.02	5	.000070*	8.73			
Error	130	.000022	19.35	130	.000030	28.96	175	.000025	26.00
Total nitrogen:									
Variety	13	.025019**	16.92	26	.015531**	16.44	35	.000494**	16.83
Year	2	.045959**	9.29	1	.100147**	8.18	5	.260941**	33.34
Age	5	.398371**	43.24	5	.478714**	40.01			
Variety × year	26	.001910*	6.83	26	.001581	5.24			
Variety × age	65	.002150**	11.45	130	.000916	8.93			
Year × age	10	.007240**	8.24	5	.013857	6.81			
Error	130	.001273	12.47	130	.002220	13.90	175	.002055	17.50
Acid-hydrolyzable polysaccharides:									
Variety	13	73.400**	72.34	26	37.116**	58.90	35	17.881**	59.94
Year	2	3.213	5.94	1	25.430**	9.57	5	65.396**	43.32
Age	5	66.718**	42.77	5	85.962**	39.31			
Variety × year	26	2.196	17.70	26	1.140	10.32			
Variety × age	65	7.158**	50.51	130	3.560**	40.79			
Year × age	10	.931	7.14	5	6.554**	10.85			
Error	130	1.453	32.18	130	.988	21.49	175	2.779	32.84
Weight of fruit:									
Variety	13	66.936**	60.27	26	35.626**	55.13	35	19.444**	54.89
Year	2	12.024**	10.02	1	10.882**	5.98	5	178.242**	62.81
Age	5	172.429**	60.00	5	239.059**	61.44			
Variety × year	26	1.853**	14.18	26	.891	8.72			
Variety × age	65	6.169**	40.92	130	3.717**	39.82			
Year × age	10	2.219**	9.62	5	2.599**	6.53			
Error	130	.797	20.70	130	.725	17.59	175	2.459	43.64

*Significant; **highly significant.

The data show highly significant variations attributable to differences in varieties and age. The mean squares that are significant in the 3-year group of varieties are generally also significant in the 2-year and 1-year groups. The variation due to differences in years is in general relatively small and in a few cases not significant. For most constituents the age of the fruit is the source of greater variation than variety. There is considerable unexplained variance, obviously due to sampling error, resulting from the large differences in the individual fruits. The acid-hydrolyzable polysaccharides are generally the most variable of the chemical constituents determined, the variation being even greater in many cases than that of the total fresh weight of the fruit. Other constituents whose variability appears to be large and important are total solids and total sugars. The variability of the content of acids, astringent materials, soluble solids, and reducing sugars is in most cases smaller, for both principal factors and their interactions, than that of the other constituents.

RELATION OF WEIGHT AND CHEMICAL CONSTITUENTS TO AGE

In order to obtain an understanding of the general relation between the age and size of the fruit and its composition, the results at each stage of maturity and each storage period for all varieties and all years were averaged. The curves up to and including 60 days from date of bloom (fig. 1) are means for a series of 79 samples. Six of these were discontinued at various times during the storage period, so that the points on the curves at 90, 120, and 165 days are means for 77, 75, and 73 samples, respectively. It is obvious that the various curves could not have been measurably different had the entire series been carried to the end of the storage period.

The increase in the fresh weight of the fruit is represented by a curve (fig. 1) similar to the general growth curve. The maximum fresh weight of the fruit was reached at about 60 days of age, after which there was a slow decrease in storage. Because of the rather high humidity and moderately low temperature of the storage room this decrease in weight caused by drying was small. The respiration was evidently fairly rapid, since total solids decreased more rapidly than the fresh weight. The decrease in total solids of the fresh material began before the fruit was harvested, was greatly accelerated in the first month of storage (60 to 90 days of age), and then slowed down. About two-thirds of the total loss in storage occurred in the first third of the storage period.

As would be expected, the course of the chemical changes of the several constituents differed greatly. The changes in no constituent exactly paralleled those in any other, but in several constituents they were somewhat similar. The direction of the curves for acidity, total astringency, and total nitrogen is very similar. The curves for soluble solids and total sugar are nearly parallel except during the first 10 days' growth, when the changes in the sugar were pronounced and the soluble solids changed little. During the same period the total solids decreased, while the acid-hydrolyzable polysaccharides increased; for the remainder of the life of the fruit the direction of the changes in the two curves was very similar. The curves indicate that the acid-hydrolyzable polysaccharides reached a maximum somewhat earlier than the total solids and the fresh weight. The direction of the curve for total nitrogen is almost the reverse of that for nitrate

nitrogen; that is, when the total nitrogen increased, the nitrate nitrogen decreased.

The changes in most of the constituents during the growing period, especially up to the 40-day stage, were much more pronounced than those that took place during storage. The most pronounced change in any constituent during storage was in the acid-hydrolyzable poly-
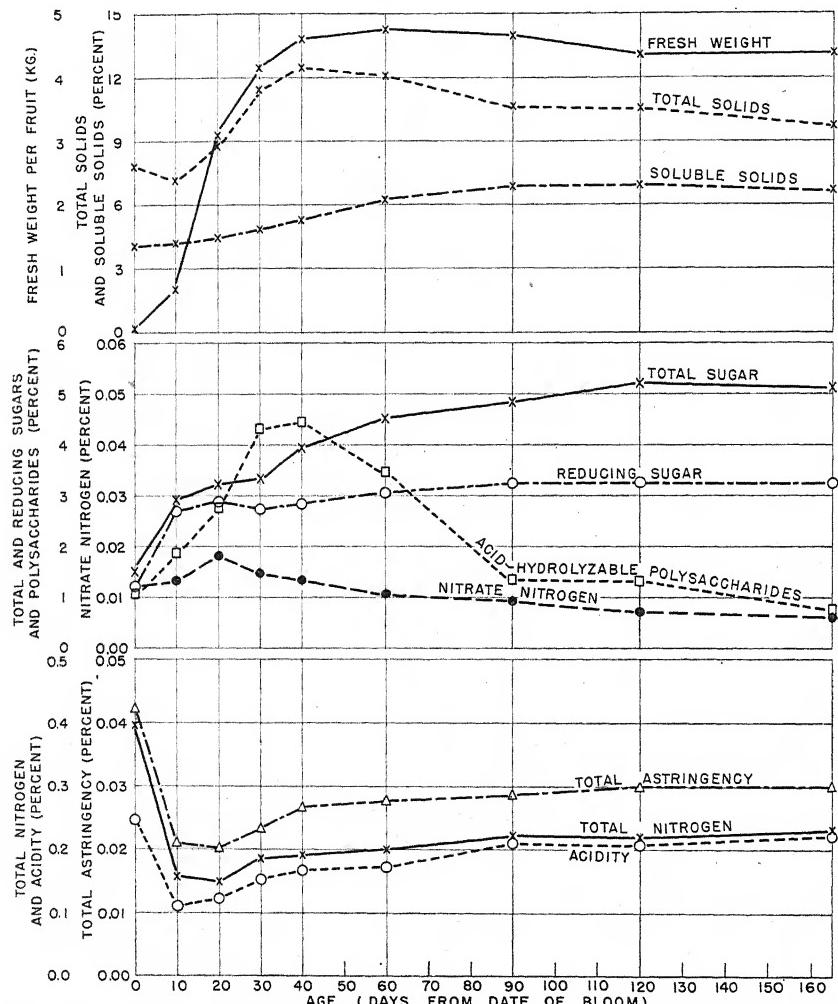
FIGURE 1.—General relation between the fresh weight, or size, of *Cucurbita* fruits, their content (fresh-weight basis) of various chemical constituents, and their age.

saccharides. Next in importance was the change in total sugar. Total sugar increased while acid-hydrolyzable polysaccharides decreased. Total nitrogen and astringent substances increased somewhat during storage as a result of greater loss of other constituents, whereas nitrate nitrogen decreased.

The changes in the composition are of such importance in relation to the problems connected with the use of the fruit as food and in

studies of the physiological processes that it seemed advisable to compare the results expressed on the fresh-weight basis with those expressed on the dry-weight basis. The results have therefore been calculated as percentages of the dry matter present.

The curves on the dry-weight basis for the soluble solids, total sugar, and reducing sugars are somewhat similar; that is, they increase from flowering to the 10-day stage and then decrease to the 30- or 40-day stage, after which they increase to the end of the storage
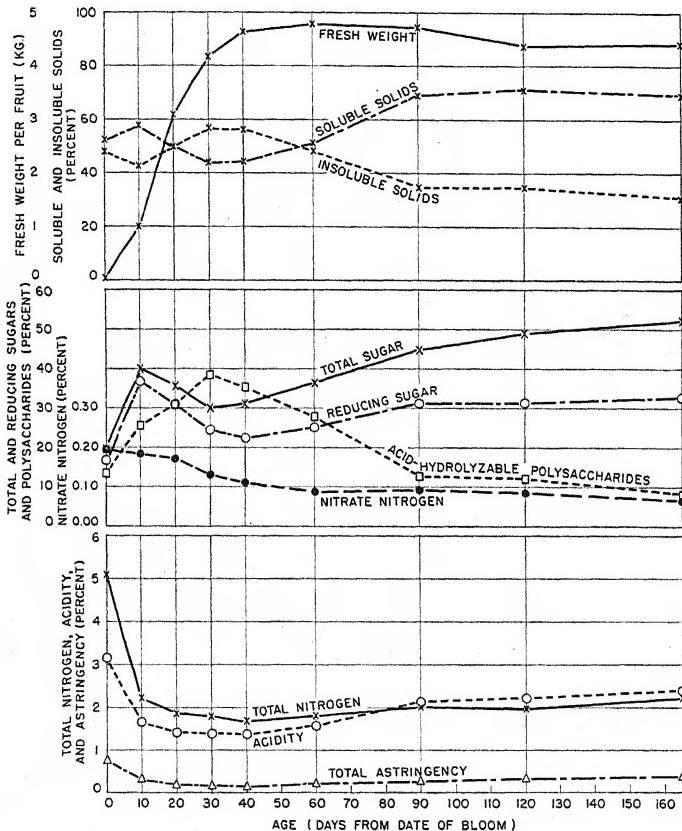
FIGURE 2.—General relation between the fresh weight, or size, of *Cucurbita* fruits, their content (dry-weight basis) of various chemical constituents, and their age.

period (fig. 2). The decrease between the 10- and the 30-day stages is due very largely to the increase in the acid-hydrolyzable polysaccharides during this period. These curves are rather strikingly different from those on the fresh-weight basis, as is evident from a comparison of figures 1 and 2. The sugars and soluble solids are lowest at the 30- and 40-day stages, when the acid-hydrolyzable polysaccharides are highest. It is evident that the starch-sugar ratio is highest at this period.

The curves for total and nitrate nitrogen and acidity are somewhat similar to the corresponding curves on the fresh-weight basis. In most cases the changes that occur before maximum fresh weight has

been attained are much more rapid and more pronounced than those that take place subsequently to or during storage.

While the percentage composition is of great importance because of its relation to the edible quality and food value of the product, the yield, or the total amount of the constituents produced, determines to an even greater extent the value of the crop and must be given con-
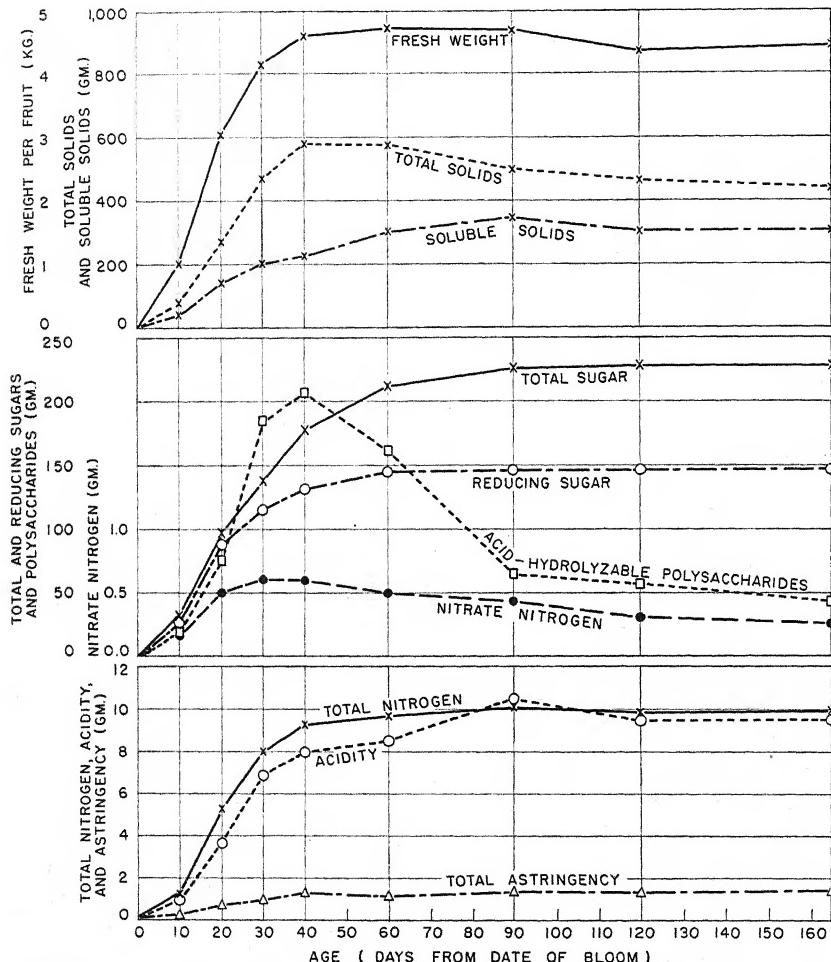
FIGURE 3.—General relation between the fresh weight, or size, of *Cucurbita* fruits, the total amounts per fruit of various chemical constituents. and their age.

sideration. Consequently, it seemed desirable to study the relation of the age of the material to the total amounts of the constituents produced. The data obtained afford an idea of the comparative rates of change in the total amounts of the constituents per fruit. The fresh-weight per fruit was multiplied by the percentage of each of the constituents determined in the analyses and the results were plotted (fig. 3).

The curves in figure 3 show the total amount of each constituent per fruit if one assumes that the entire fruit consists of wall tissue. Since the wall tissue makes up such a large part of the fruit, these curves probably correspond rather closely to those for actual values. All the curves except those for total solids and acid-hydrolyzable polysaccharides are somewhat similar, rising slowly from the flowering to the 10-day stage, very rapidly to the 30- or 40-day stage, and then progressively more slowly to the 40- or 60-day stage. The curve for total solids is almost a straight line from the 10-day to the 40-day stage; that is, for the greater part of the period of growth of the fruit. Consequently, it is evident that dry matter is added to the fruit at an almost constant rate over the greater part of the growing period. During the storage period, that is, from the 60-day stage onward, there are only moderate or inconsequential changes in the total amount per fruit of soluble solids, total sugars, total nitrogen, and acidity. All of these, except soluble solids, which decrease toward the end of the storage period, tend to increase slightly. The greatest change in the total amount per fruit during storage was in the acid-hydrolyzable polysaccharide content. The change in the total-solids content was also quite marked. There was a small but important decrease in fresh weight and a definite though less pronounced decrease in nitrate nitrogen.

RANKING OF VARIETIES ACCORDING TO CONTENT OF THEIR CHEMICAL CONSTITUENTS

In the discussion of the analysis of variance it was pointed out that there are large and highly significant variations attributable to differences among varieties. To determine the general rank of the different varieties, the variety means were obtained by averaging the results at all stages of maturity for all years. These average values are much more reliable for general comparison than those obtained only at the time of harvest. However, the ranking is essentially the same in both cases. The comparisons of the varieties for the different average constituents are shown in table 3.

TABLE 3.—*Rank of 36 Cucurbita varieties according to the average content of various constituents (fresh-weight basis)*

Constituent, numerical rank, and variety	Content	Constituent, numerical rank, and variety	Content
	Percent		*Percent*
Total solids:		Total solids—Continued.	
1. Table Queen	15.7	21. French Turban	9.1
2. Delicious	15.2	22. Japanese Pie	9.0
3. Banana	13.4	23. Golden Oblong	8.8
4. Improved Hubbard	13.1	24. New England Pie	8.7
5. Sibley	12.9	25. Marblehead	8.2
6. Golden Hubbard	12.9	26. Green Striped Cushaw	8.1
7. Fordhook	12.8	27. Fort Berthold	7.9
8. Blue Hubbard	12.3	28. Mammoth Golden Cushaw	7.8
9. Winnebago	12.1	29. Large Cheese	7.6
10. Chicago Warted Hubbard	11.8	30. Mammoth Chili	7.5
11. Gilmore	11.7	31. Omaha	7.4
12. Essex Hybrid	11.5	32. Winter Luxury	7.3
13. Kitchenette	11.4	33. Mammoth Whale	7.3
14. Golden Delicious	10.8	34. Faan Kwa	7.0
15. Arikara	10.5	35. Connecticut Field	6.7
16. Iron Bark	10.4	36. King of the Mammoths	6.4
17. Warren	10.1	Soluble solids:	
18. Tennessee Sweet Potato	9.4	1. Table Queen	8.1
19. Boston Marrow	9.2	2. Fordhook	7.2
20. Charles Naudin	9.1	3. Sibley	6.6

TABLE 3.—*Rank of 36 Cucurbita varieties according to the average content of various constituents (fresh-weight basis)*—Continued

Constituent, numerical rank, and variety	Content	Constituent, numerical rank, and variety	Content
Soluble solids—Continued.	*Percent*	Acid-hydrolyzable polysaccharides— Con.	*Percent*
4. Delicious	6.4	8. Blue Hubbard	3.5
5. Chicago Warted Hubbard	6.3	9. Essex Hybrid	3.5
6. Improved Hubbard	6.25	10. Kitchenette	3.4
7. Winnebago	6.2	11. Winnebago	3.2
8. Blue Hubbard	6.2	12. Gilmore	3.0
9. Gilmore	6.1	13. Fordhook	3.0
10. Iron Bark	6.0	14. Arikara	2.9
11. Golden Hubbard	5.9	15. Chicago Warted Hubbard	2.7
12. Kitchenette	5.8	16. Iron Bark	2.6
13. Golden Delicious	5.8	17. Warren	2.5
14. Essex Hybrid	5.7	18. French Turban	2.4
15. Golden Oblong	5.7	19. Boston Marrow	2.0
16. Warren	5.6	20. Tennessee Sweet Potato	1.9
17. New England Pie	5.6	21. Japanese Pie	1.8
18. Charles Naudin	5.5	22. Charles Naudin	1.6
19. Tennessee Sweet Potato	5.5	23. Green Striped Cushaw	1.8
20. Arikara	5.4	24. Marblehead	1.8
21. Japanese Pie	5.4	25. Mammoth Whale	1.6
22. Boston Marrow	5.3	26. Mammoth Chili	1.6
23. Omaha	5.2	27. Mammoth Golden Cushaw	1.5
24. French Turban	5.0	28. Large Cheese	1.4
25. Fort Berthold	5.0	29. New England Pie	1.3
26. Mammoth Golden Cushaw	4.9	30. Faan Kwa	1.1
27. Marblehead	4.9	31. Golden Oblong	1.1
28. Mammoth Whale	4.9	32. Omaha	1.0
29. Large Cheese	4.8	33. Fort Berthold	1.0
30. Green Striped Cushaw	4.8	34. Winter Luxury	1.0
31. Winter Luxury	4.7	35. King of the Mammoths	.9
32. Faan Kwa	4.6	36. Connecticut Field	.6
33. Mammoth Chili	4.6	Total nitrogen:	
34. King of the Mammoths	4.6	1. Delicious	.298
35. Mammoth Whale	4.4	2. Fordhook	.277
36. Connecticut Field	4.4	3. Table Queen	.271
Total sugars:		4. Kitchenette	.270
1. Table Queen	6.0	5. Golden Hubbard	.259
2. Fordhook	5.3	6. Banana	.258
3. Sibley	4.9	7. Gilmore	.251
4. Iron Bark	4.6	8. Blue Hubbard	.250
5. Winnebago	4.4	9. Golden Delicious	.249
6. Chicago Warted Hubbard	4.3	10. Improved Hubbard	.248
7. Delicious	4.3	11. Arikara	.237
8. Blue Hubbard	4.3	12. Chicago Warted Hubbard	.235
9. Banana	4.2	13. Winnebago	.235
10. Gilmore	4.2	14. Essex Hybrid	.230
11. Improved Hubbard	4.1	15. Sibley	.226
12. Japanese Pie	4.0	16. Iron Bark	.214
13. Golden Oblong	4.0	17. Warren	.212
14. Tennessee Sweet Potato	4.0	18. Boston Marrow	.211
15. Kitchenette	4.0	19. Charles Naudin	.211
16. Charles Naudin	3.9	20. Japanese Pie	.205
17. Golden Hubbard	3.9	21. Marblehead	.204
18. Warren	3.8	22. Tennessee Sweet Potato	.203
19. Essex Hybrid	3.8	23. Omaha	.195
20. New England Pie	3.7	24. New England Pie	.192
21. Golden Delicious	3.6	25. French Turban	.190
22. Arikara	3.5	26. Golden Oblong	.183
23. Fort Berthold	3.5	27. Mammoth Golden Cushaw	.182
24. Omaha	3.5	28. Winter Luxury	.177
25. Boston Marrow	3.4	29. Green Striped Cushaw	.175
26. Green Striped Cushaw	3.4	30. King of the Mammoths	.170
27. French Turban	3.4	31. Mammoth Whale	.169
28. Mammoth Golden Cushaw	3.3	32. Faan Kwa	.167
29. Faan Kwa	3.3	33. Fort Berthold	.164
30. Large Cheese	3.3	34. Large Cheese	.163
31. Marblehead	3.3	35. Mammoth Chili	.162
32. Mammoth Chili	3.2	36. Connecticut Field	.148
33. King of the Mammoths	3.2	Nitrate nitrogen:	
34. Winter Luxury	3.0	1. Marblehead	.0236
35. Connecticut Field	2.9	2. Chicago Warted Hubbard	.0224
36. Mammoth Whale	2.9	3. Kitchenette	.0184
Acid-hydrolyzable polysaccharides:		4. Boston Marrow	.0169
1. Delicious	6.5	5. Sibley	.0160
2. Table Queen	4.7	6. Arikara	.0157
3. Banana	4.5	7. Mammoth Chili	.0144
4. Golden Hubbard	4.2	8. King of the Mammoths	.0143
5. Improved Hubbard	4.0	9. Golden Delicious	.0140
6. Sibley	3.7	10. Charles Naudin	.0137
7. Golden Delicious	3.7	11. Mammoth Golden Cushaw	.0133

TABLE 3.—*Rank of 36 Cucurbita varieties according to the average content of various constituents (fresh-weight basis)*—Continued

Constituent, numerical rank, and variety	Content	Constituent, numerical rank, and variety	Content
Nitrate nitrogen—Continued.	*Percent*	Acids—Continued.	*Percent*
12. Faan Kwa	0.0132	25. Japanese Pie	0.172
13. Gilmore	.0127	26. Mammoth Golden Cushaw	.172
14. Blue Hubbard	.0126	27. Green Striped Cushaw	.168
15. Essex Hybrid	.0123	28. Warren	.163
16. Mammoth Whale	.0115	29. Fort Berthold	.162
17. French Turban	.0113	30. Marblehead	.161
18. Winnebago	.0111	31. Connecticut Field	.160
19. Golden Hubbard	.0110	32. French Turban	.156
20. Fort Berthold	.0107	33. Boston Marrow	.155
21. Delicious	.0105	34. Mammoth Chili	.127
22. Iron Bark	.0105	35. King of the Mammoths	.126
23. Connecticut Field	.0104	36. Mammoth Whale	.121
24. Improved Hubbard	.0101	Total astringency:	
25. Banana	.0100	1. Banana	.034
26. Warren	.0100	2. Fordhook	.034
27. Green Striped Cushaw	.0092	3. Gilmore	.033
28. New England Pie	.0084	4. Improved Hubbard	.033
29. Omaha	.0084	5. Delicious	.032
30. Large Cheese	.0076	6. Golden Hubbard	.032
31. Winter Luxury	.0073	7. Table Queen	.032
32. Japanese Pie	.0072	8. Blue Hubbard	.031
33. Tennessee Sweet Potato	.0066	9. Arikara	.030
34. Golden Oblong	.0065	10. Kitchenette	.030
35. Table Queen	.0050	11. Essex Hybrid	.030
36. Fordhook	.0042	12. Large Cheese	.030
Acids:		13. Sibley	.030
1. Table Queen	.261	14. Chicago Warted Hubbard	.030
2. Fordhook	.253	15. Golden Delicious	.029
3. Delicious	.213	16. Winnebago	.029
4. Banana	.210	17. French Turban	.028
5. Improved Hubbard	.209	18. Warren	.028
6. Chicago Warted Hubbard	.209	19. Iron Bark	.028
7. Iron Bark	.205	20. New England Pie	.028
8. Golden Hubbard	.204	21. Omaha	.028
9. Kitchenette	.200	22. Golden Oblong	.027
10. Winnebago	.200	23. Japanese Pie	.027
11. Omaha	.195	24. Marblehead	.027
12. Charles Naudin	.195	25. Boston Marrow	.026
13. Arikara	.192	26. Charles Naudin	.026
14. Blue Hubbard	.191	27. Faan Kwa	.026
15. Sibley	.187	28. Winter Luxury	.026
16. Essex Hybrid	.185	29. Tennessee Sweet Potato	.025
17. Gilmore	.185	30. Mammoth Whale	.024
18. New England Pie	.184	31. Connecticut Field	.023
19. Winter Luxury	.181	32. Fort Berthold	.023
20. Tennessee Sweet Potato	.180	33. Green Striped Cushaw	.023
21. Golden Oblong	.177	34. Mammoth Chili	.023
22. Faan Kwa	.177	35. Mammoth Golden Cushaw	.023
23. Golden Delicious	.176	36. King of the Mammoths	.022
24. Large Cheese	.176		

TOTAL SOLIDS

Table 3 shows a wide range in the total-solids content of the different varieties, the extremes being 6.4 and 15.7 percent of the fresh weight. About half of the group fall within the range of 8 to 12 percent solids.

Among those that rank high in total solids are a number of widely grown varieties, such as Table Queen, Delicious, Banana, Improved Hubbard, Sibley, and Golden Hubbard; among those low in total solids are King of the Mammoths, Connecticut Field, Mammoth Whale, Winter Luxury, Omaha, and Mammoth Chili, all widely grown. The species *Cucurbita pepo* contains some varieties that are among the highest in solids and others that are among the lowest, Table Queen and Fordhook being high and Connecticut Field and Winter Luxury being low. Among the varieties of the species *C. moschata* none were outstandingly high in total solids, but Faan

Kwa and Large Cheese were among the lowest. In the *C. maxima* group Improved Hubbard and Banana were high, while King of the Mammoths and Mammoth Chili were low.

A fairly good negative correlation was found between size of fruits at maturity and total-solids content; in other words, the large fruits were generally higher in moisture than the small fruits.

SOLUBLE SOLIDS

The ranking of the varieties for soluble solids is somewhat like that for total solids (table 3). Table Queen, Fordhook, Delicious. Improved Hubbard, and Sibley are among the highest in both total and soluble solids, whereas Connecticut Field, King of the Mammoths, Faan Kwa, Mammoth Chili, and Mammoth Whale are among the lowest.

TOTAL SUGARS AND ACID-HYDROLYZABLE POLYSACCHARIDES

The ranking of the varieties in respect to total sugar content is similar to that for acid-hydrolyzable polysaccharide content (table 3). Varieties that on an average are high in sugar are generally high in acid-hydrolyzable polysaccharides. High acid-hydrolyzable polysaccharide content in fruit at or approaching maturity is correlated with high sugar content late in the storage period. The polysaccharide content is particularly high in Delicious and Table Queen and very low in Connecticut Field, while the sugar content is particularly high in Table Queen and low in Mammoth Whale and Connecticut Field.

TOTAL NITROGEN

The varieties with a high total-solids content (table 3) were also generally somewhat higher in total nitrogen than the varieties with a low total-solids content. On the other hand, if the results are calculated on the dry-weight basis the varieties that are high in total solids are found to be low in total nitrogen. In other words, there is a smaller proportion of nitrogenous materials in the total solids of those varieties high in dry matter than in those low in dry matter, the principal constituents that account for the high total solids being sugars and acid-hydrolyzable polysaccharides.

NITRATE NITROGEN

Among the varieties low in nitrate nitrogen (table 3) are Fordhook, Table Queen, Golden Oblong, and Tennessee Sweet Potato, while among those highest in nitrates are Marblehead, Chicago Warted Hubbard, Kitchenette, and Boston Marrow. These differences are important because of the corroding effect of nitrates on tin containers (*5*).

TITRATABLE ACIDITY

Although the differences in acid content were often statistically significant, they seemed to be unimportant from a practical standpoint. Among the varieties that were highest in acidity are Table Queen, Fordhook, Delicious, Banana, Improved Hubbard, Chicago Warted Hubbard, and Golden Hubbard (table 3); Mammoth Whale, King of the Mammoths, and Mammoth Chili were exceptionally low, their acid content being on an average less than half of that of the highest.

Total Astringency

The astringency of the fruits of all the varieties was very low, and differences, although sometimes statistically significant, appear to be entirely unimportant from the standpoint of the quality of the products. Among those highest (table 3) were Banana, Fordhook, Gil-
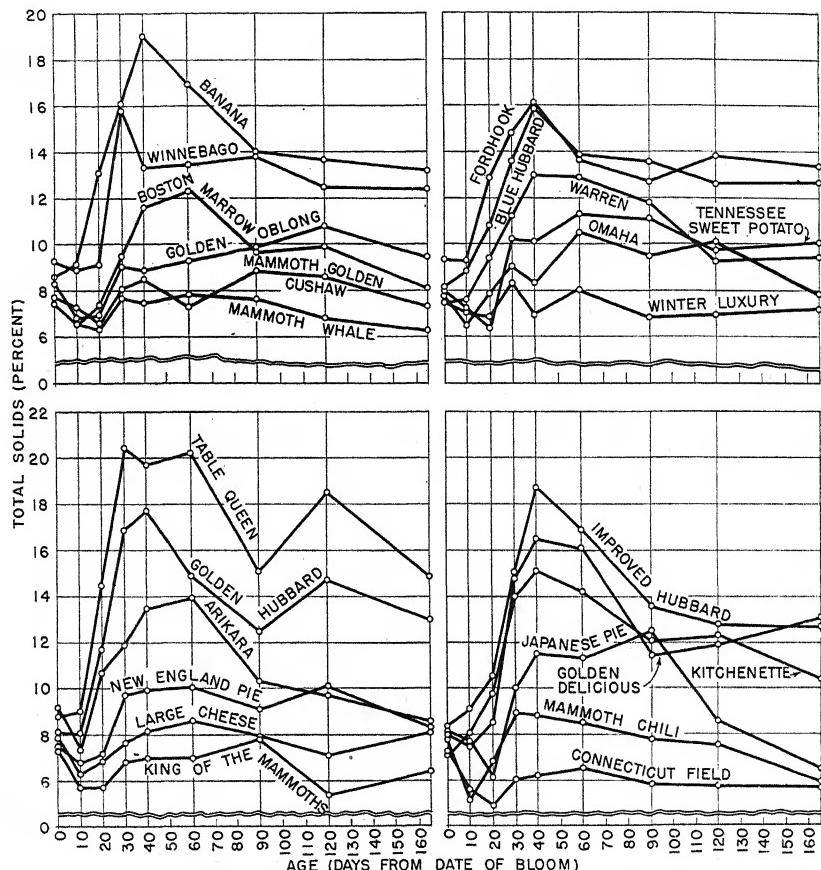
FIGURE 4.—Relation between the average total-solids content of the fruits of some *Cucurbita* varieties and their age, showing how the changes that occur differ in the different varieties.

more, and Improved Hubbard, the last being more widely grown than the others.

VARIETAL DIFFERENCES AT DIFFERENT AGES

Although the means shown in table 3 indicate the general ranking of the varieties as to composition, it is often more important to know how they rank at a particular stage of maturity or storage. From table 2 it is apparent that there is a rather large, highly significant variance attributable to the interaction of variety and age. This variance (variety $\times$ age) shows that the varieties do not differ to the same extent at all stages of maturity.

Total Solids

The changes in the percentage of total solids in the fresh material with increase in age are comparatively small in certain varieties and rather large in others (fig. 4). The percentages of total solids in all varieties are nearly the same at the time of flowering. In Mammoth Whale, Winter Luxury, King of the Mammoths, Mammoth Golden Cushaw, and Connecticut Field they change relatively little during the entire period of growth and throughout storage. On the other hand,
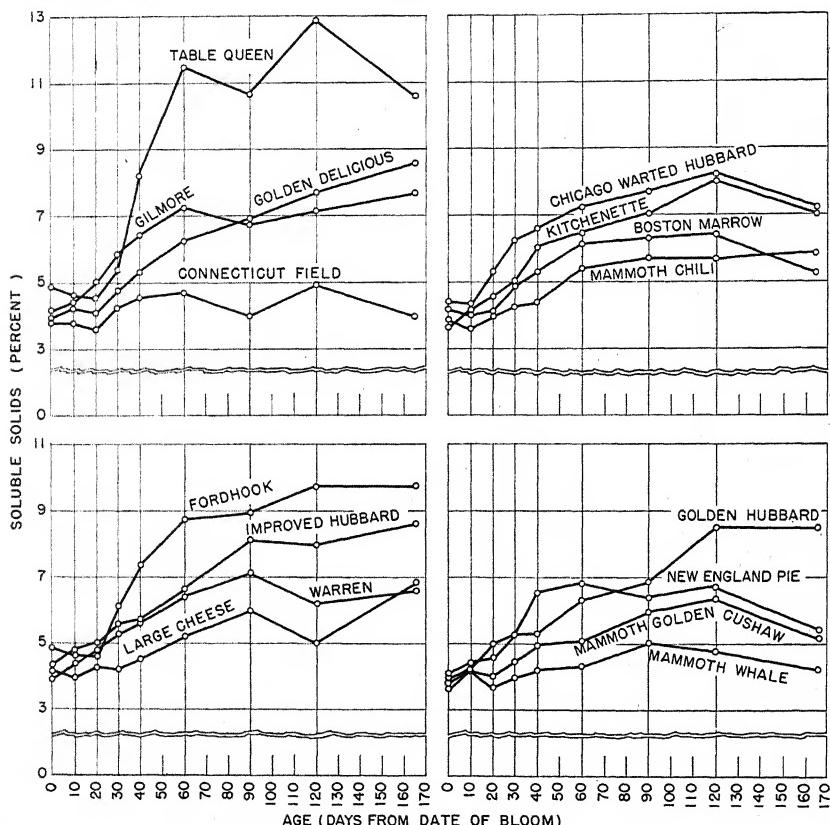
FIGURE 5.—Relation between the average soluble-solids content of the fruits of some *Cucurbita* varieties and their age, showing how the changes that occur differ in different varieties.

the total solids (percent) in Table Queen, Fordhook, Banana, Improved Hubbard, Blue Hubbard, and Golden Delicious increase tremendously from the flowering stage to the 30- or 40-day stage, but they decline slowly during storage. Between these two groups there are a number of varieties that are intermediate in the extent of the changes during development.

Soluble Solids

The differences among the varieties in respect to changes in soluble solids are less striking than in total solids, but the differences are often important (fig. 5), as in the case of Connecticut Field and Table

Queen. There were comparatively small changes in Mammoth Whale and Mammoth Chili with increase in age, but rather large changes occurred in Fordhook, Chicago Warted Hubbard, Table Queen, and Golden Hubbard. New England Pie, Boston Marrow, and Mammoth Whale, showed a tendency to decrease in soluble
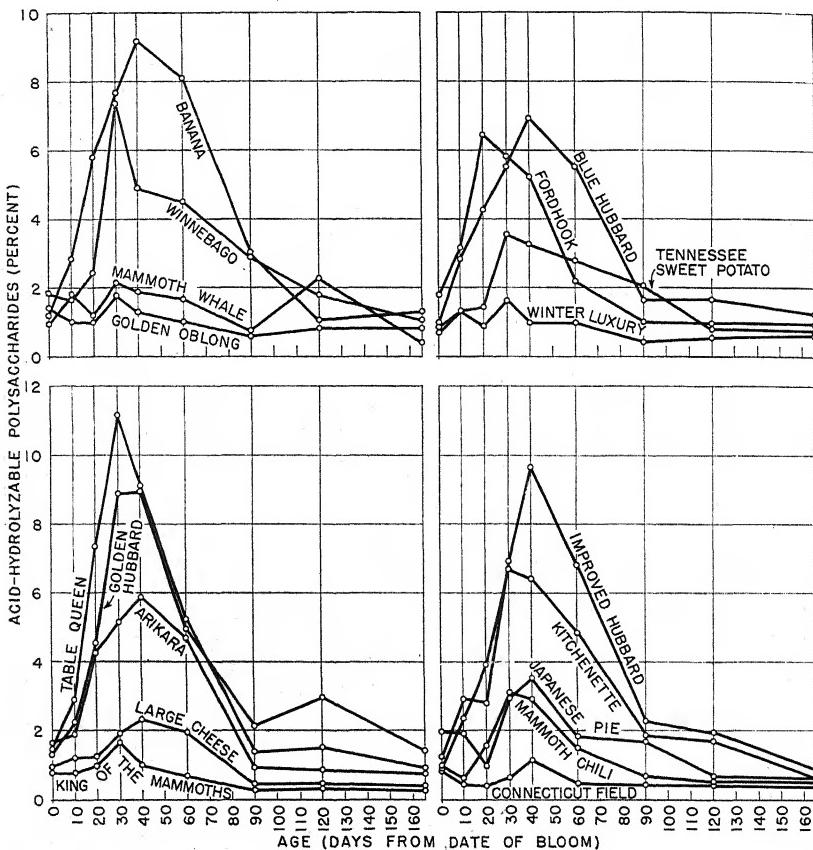
FIGURE 6.—Relation between the average acid-hydrolyzable polysaccharides content of the fruits of some *Cucurbita* varieties and their age, showing how the changes that occur differ in different varieties.

solids during storage, whereas Golden Delicious, Golden Hubbard, Improved Hubbard, and Fordhook showed a tendency to increase.

ACID-HYDROLYZABLE POLYSACCHARIDES

The variance attributable to the interaction of age with variety is greater in the acid-hydrolyzable polysaccharides than in any other constituent of the total solids. This is accounted for by the differences in the changes of the varieties with increase in age, which is often very striking (fig. 6). Examples of this extreme contrast are Banana and Golden Oblong, Table Queen and King of the Mammoths, Blue Hubbard and Winter Luxury, Improved Hubbard and Connecticut

Field. Between the varieties that are highest and those that are lowest are others of intermediate type, among which may be mentioned Tennessee Sweet Potato, Arikara, Japanese Pie, and Mammoth Chili.

TOTAL SUGARS

Since sugar content at the time of flowering is very low, the differences in varieties at this stage are comparatively small (fig. 7).
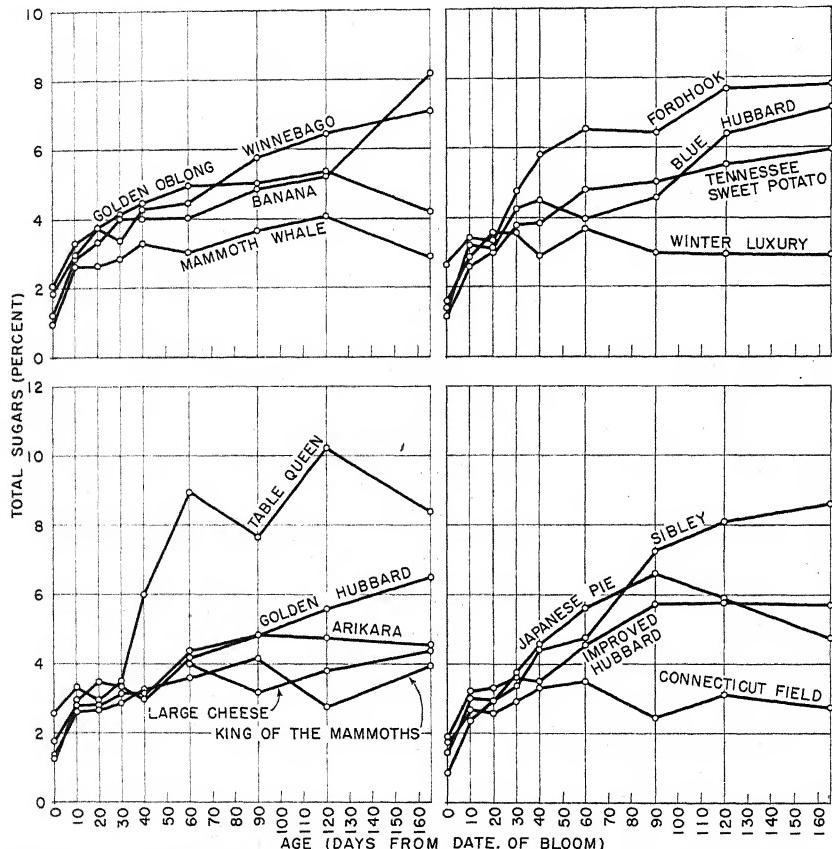
FIGURE 7.—Relation between the average sugar content of the fruits of some *Cucurbita* varieties and their age, showing how the changes that occur differ in different varieties.

With increasing age, however, the differences between varieties may become much more pronounced. Certain varieties, such as Connecticut Field, Winter Luxury, Mammoth Whale, and King of the Mammoths, never become very high in sugar, whereas other varieties, such as Table Queen, Fordhook, Banana, and Blue Hubbard, are much higher than the average in the older stored material (90 to 120 days of age). There is generally a similar increase in sugar in all varieties up to the 30- or 40-day stage, after which the low-sugar varieties cease to increase while the others continue to increase far

into the storage period. The reason for this is obvious: those varieties that are low in acid-hydrolyzable polysaccharides do not increase greatly in sugar during storage, while those high in acid-hydrolyzable polysaccharides eventually become high in sugar as a result of the conversion of starch into sugar.

Total and Nitrate Nitrogen, Acidity, and Astringency

The variance attributable to the interaction of age and variety in the case of the other consitituents is generally not as significant or as
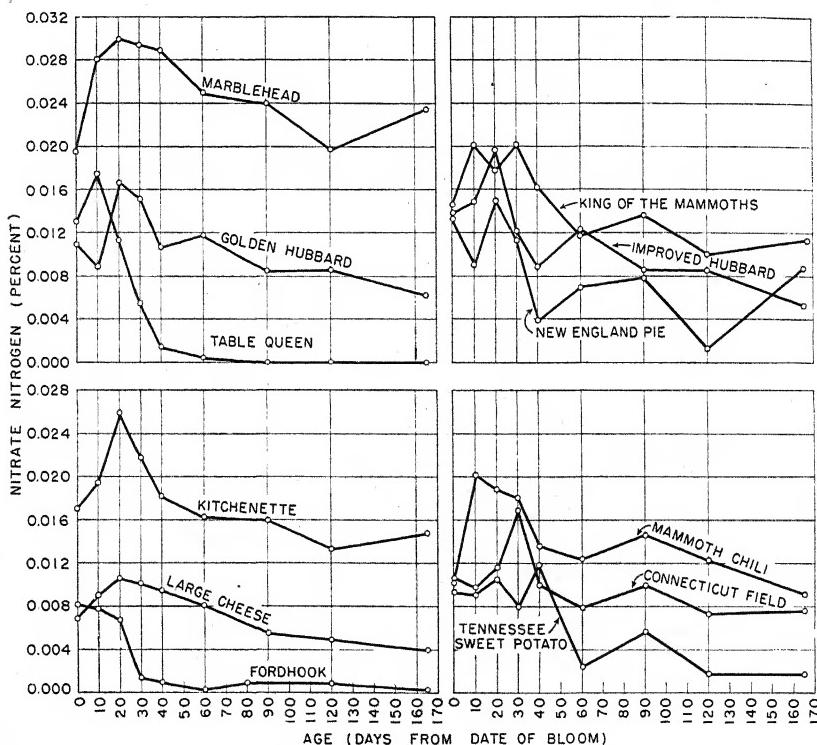
FIGURE 8.—Relation between the average nitrate nitrogen content of the fruit of some *Cucurbita* varieties and their age, showing how the changes that occur differ in different varieties.

important as with the acid-hydrolyzable polysaccharides, sugars, and total solids. It does appear to be important in the case of nitrate nitrogen (fig. 8) in a few varieties. Table Queen and Fordhook, for example, have appreciable amounts of nitrate nitrogen in their tissues only in the early developmental stages. From about the 40-day stage onward nitrates are negligible in these varieties, whereas in Marblehead, Golden Hubbard, and Kitchenette appreciable quantities exist far into the storage period.

The changes in total nitrogen (fig. 9) in the different varieties differ somewhat, but the results are too irregular to be of much significance.

The changes in acidity are also somewhat greater in certain varieties than in others as is evident from a comparison of Table Queen and King of the Mammoths (fig. 10).

The differences between varieties in the changes in astringency are generally not very important, as is evident from a comparison of the
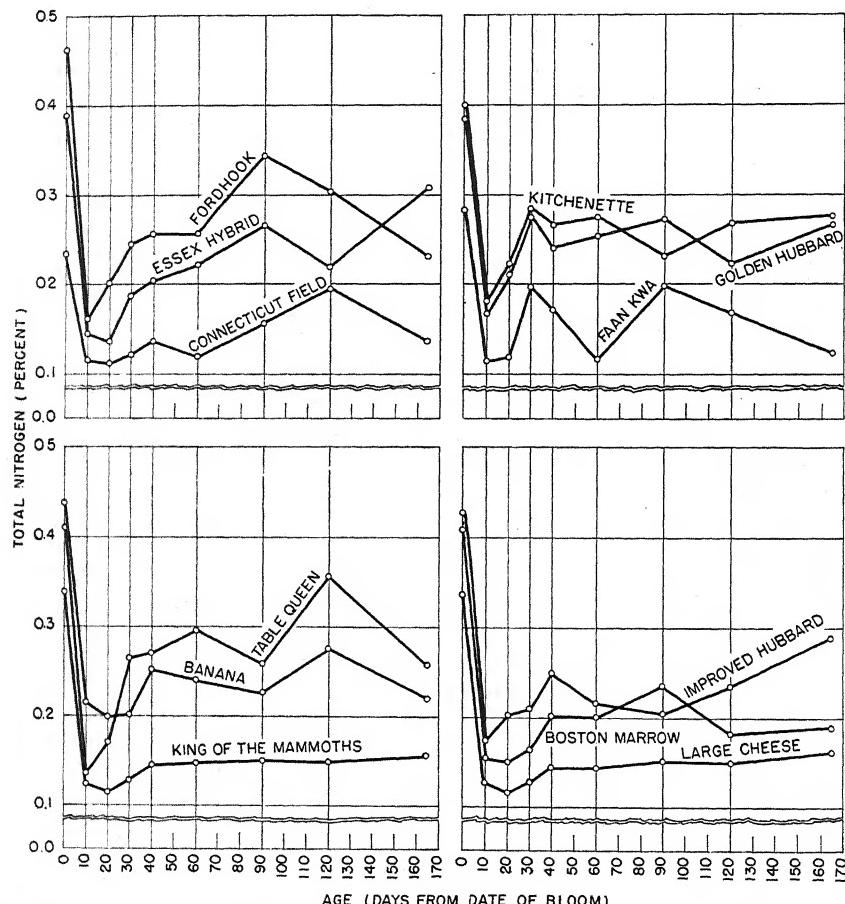

FIGURE 9.—Relation between the average total nitrogen content of the fruits of some *Cucurbita* varieties and their age, showing how the changes that occur differ in different varieties.

curves (fig. 11) for Fordhook and Connecticut Field, which show about the extreme differences.

COMPOSITION AND CULINARY USE

It is apparent from the reports of several workers (*6, 7, 8, 11, 13, 16*) that many differences in the taste and physical character of *Cucurbita* varieties and their adaptability to different uses are directly attributable to differences in their composition.

Numerous cooking and canning tests have been made with the material used in these analyses. Pumpkins are usually canned soon

after harvest or at about the stage of maturity of the material analyzed at 60 days of age. An examination of the material canned at this stage revealed wide differences in the color, flavor, and consistency of the canned products of different varieties. The ratings of the canned products (table 4) illustrate the way in which the varieties may differ. Some varieties are suited for one purpose and some for another; hence those considered best may differ greatly, depending upon the use to which they are to be put and the method of preparing them for this use.

TABLE 4.—*Rank of 36 Cucurbita varieties based on the color, flavor, and consistency of the canned product*

[Each value is an average for 3 years; the lowest value indicates the most desirable product in each case]

Color		Flavor		Consistency [1]	
Variety	Rating	Variety	Rating	Variety	Penetrometer reading
					Mm.
Boston Marrow	1.0	Golden Delicious	1.0	Delicious	17.2
Golden Delicious	1.3	Boston Marrow	1.0	Banana	21.2
Golden Hubbard	1.3	Golden Hubbard	1.3	Improved Hubbard	21.3
Essex Hybrid	1.5	Essex Hybrid	1.5	Blue Hubbard	21.3
Warren	2.5	Warren	1.5	Kitchenette	24.0
New England Pie	3.0	Delicious	1.5	Iron Bark	25.7
Winter Luxury	3.0	Improved Hubbard	2.3	Golden Delicious	25.8
Fort Berthold	3.0	Table Queen	2.3	Winnebago	26.0
Mammoth Golden Cushaw	3.0	Winnebago	2.3	Golden Hubbard	26.1
Golden Oblong	3.3	Blue Hubbard	2.5	Essex Hybrid	26.3
Gilmore	3.5	Chicago Warted Hubbard	3.0	Chicago Warted Hubbard	26.5
King of the Mammoths	3.5	Kitchenette	3.0	Gilmore	27.0
Omaha	4.0	Sibley	3.5	Warren	28.4
Delicious	4.0	Banana	3.5	Table Queen	28.6
Banana	4.5	Fordhook	3.7	Marblehead	28.7
Large Cheese	4.7	Gilmore	4.0	Sibley	28.8
Mammoth Chili	5.0	King of the Mammoths	4.5	Boston Marrow	31.1
Arikara	5.6	Mammoth Chili	4.5	Fordhook	31.2
Improved Hubbard	5.6	Arikara	4.6	Arikara	31.6
Blue Hubbard	6.0	New England Pie	5.0	Omaha	32.1
Kitchenette	6.0	Charles Naudin	5.2	Charles Naudin	32.3
Marblehead	6.0	Omaha	5.3	French Turban	32.5
Chicago Warted Hubbard	6.0	Golden Oblong	5.3	Tennessee Sweet Potato	33.2
Winnebago	6.5	Fort Berthold	5.5	Green Striped Cushaw	34.1
Table Queen	6.6	Winter Luxury	5.5	New England Pie	34.2
Faan Kwa	7.0	Mammoth Whale	5.5	Mammoth Whale	34.3
Fordhook	7.0	Marblehead	5.7	Golden Oblong	34.8
Japanese Pie	7.0	Iron Bark	6.5	Japanese Pie	35.7
Iron Bark	7.5	Connecticut Field	6.6	Fort Berthold	35.7
Sibley	7.5	French Turban	7.0	Winter Luxury	35.9
Connecticut Field	7.6	Large Cheese	7.0	Faan Kwa	36.5
French Turban	8.0	Mammoth Golden Cushaw	7.6	Mammoth Golden Cushaw	36.9
Charles Naudin	8.0	Faan Kwa	7.6	Mammoth Chili	37.9
Mammoth Whale	8.0	Japanese Pie	7.8	Large Cheese	39.4
Green Striped Cushaw	8.0	Green Striped Cushaw	8.0	Connecticut Field	40.9
Tennessee Sweet Potato	8.0	Tennessee Sweet Potato	8.2	King of the Mammoths	45.4

[1] Consistency was measured by means of a penetrometer originally devised for measuring the consistency of greases in accordance with specifications of the American Society for Testing Materials, for Test D 217–27 T (*1, pp. 421–426*). The lower the penetrometer reading, the thicker or heavier was the consistency of the product.

COLOR

The order of preference as to the color of the canned product was almost the same as that of the intensity of the yellow color of the carotenoid pigment. Sometimes there was a certain amount of darkening that detracted from the appearance of the material. Some varieties have green skins and the green color may extend into the flesh of the material; this gives the product a dull appearance. The

variety Delicious would have ranked higher had it not been for the dullness resulting from this green color. The color ranking of the varieties was nearly the same, no matter for what purpose the product was to be used. A bright, deep-yellow color was preferred in material to be boiled, cooked with butter or other seasoning, baked, fried, or made into pies. A few varieties with ratings between 1 and 1.5, such as Golden Delicious, Boston Marrow, and Golden Hubbard, were outstanding in appearance. Varieties ranking from
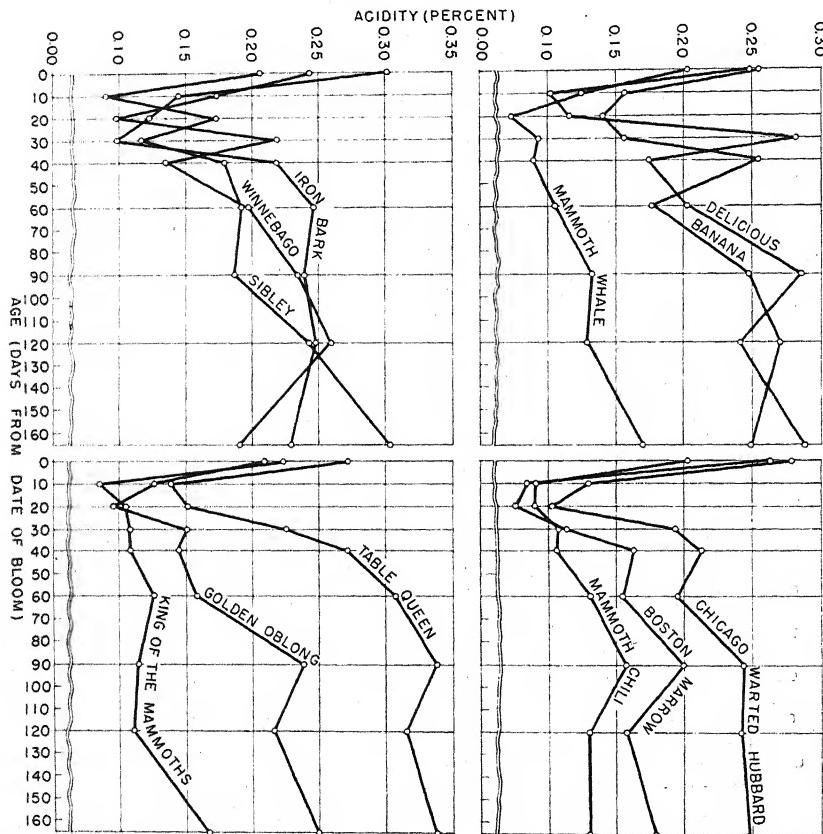

FIGURE 10.—Relation between the average acid content of the fruit of some *Cucurbita* varieties and their age, showing how the changes that occur differ in different varieties.

2.5 to 5.0, among which were New England Pie, Winter Luxury, Mammoth Golden Cushaw, and Large Cheese, were considered entirely satisfactory. Among the poorest were Mammoth Whale, Green Striped Cushaw, and Tennessee Sweet Potato, the first belonging to the *Cucurbita maxima* group and the last two to the *C. moschata* group.

FLAVOR

In contrast to the ratings for color, those for flavor varied greatly, depending upon the use to be made of the material and the preferences

of the individuals rating the varieties. In table 4 the order is based upon the flavor of the material just as it was emptied from the can. This order is practically the same as that based on the taste of the fresh material baked or steamed. The order of preference was considerably different when the material was made into pies. In many varieties the flavor of the canned or baked products was too strong
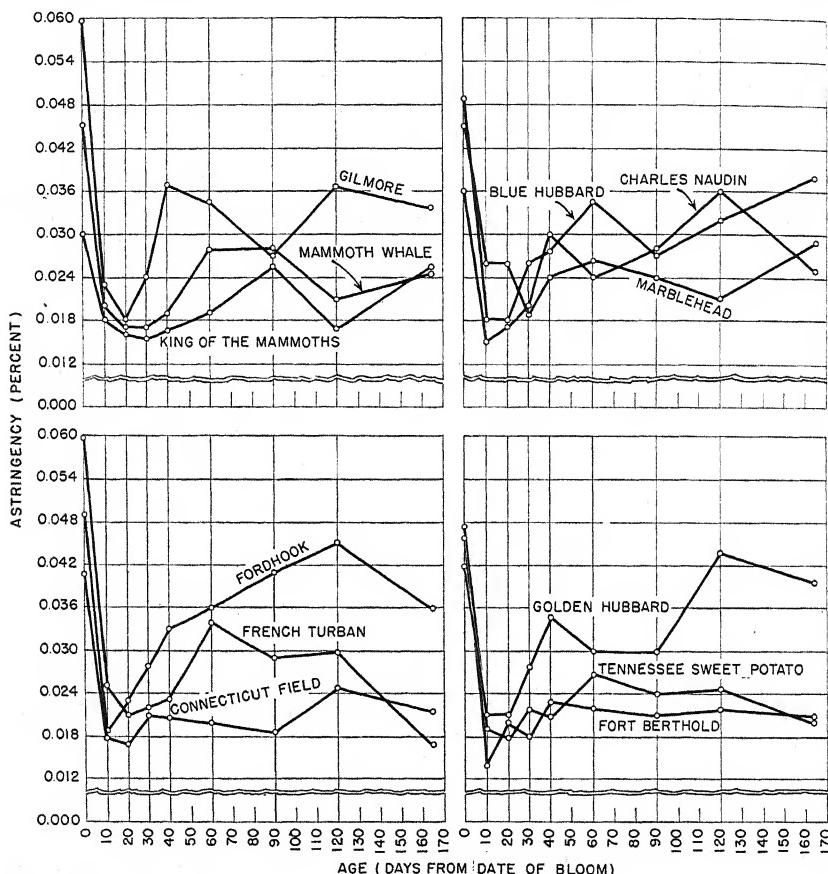

FIGURE 11.—Relation between the average total astringency content of the fruits of some *Cucurbita* varieties and their age, showing how the changes that occur differ in different varieties.

to be entirely pleasing. This was true particularly of varieties of the species *Cucurbita moschata*, such as Large Cheese and Mammoth Golden Cushaw. The flavor of these varieties was more agreeable, and hence they were rated higher in pies than when tasted directly, as in this case. The strong flavor of many varieties may account for the use of such spices as nutmeg and cinnamon in preparing table dishes of them. The varieties of the species *C. maxima* were generally much milder and were more often preferred than those of the other species. Some of the varieties of *C. pepo*, among which were Table Queen and Fordhook, were fairly mild in flavor, while others such as Winter Luxury and New England Pie, were rather strong.

The intensity of the characteristic pumpkin flavor varied greatly with the age of the material and the length of time that it remained in storage. The more mature the material or the longer in storage the stronger the flavor. The flavor of the immature material was mild, but it became much more intense between the 40- and 60-day stages and appeared to increase slowly during storage. The rapid increase in flavor in material from 30 to 60 days of age was accompanied by an increase in the brightness and apparent depth of the yellow color. It is during this period that ripening occurs.

Pleasing flavor apparently depends partly upon the sugar content. The rather high ranking of Table Queen, a *Cucurbita pepo* variety, seems to be due to the fact that it is exceptionally high in sugar. If sugar is added to Winter Luxury to make its sugar content equal to that of Table Queen, the differences in flavor between the two are greatly reduced.

Consistency

In the consistency measurements, the lowest reading indicates the heaviest, most viscous, or driest product. If the results in table 4 are compared with those in table 3, a reasonably good correlation will be found between acid-hydrolyzable polysaccharides and consistency, as well as between total solids and consistency. It is apparent that starch is more important than any other constituent in determining the consistency of the product. The cell-wall material and the pectin apparently do not vary widely and consequently do not cause great variations in the consistency, though they too are important.

Many varieties belonging to the species *Cucurbita maxima* were heavy in consistency, while a few were thin and watery; Delicious was at the top of the list and King of the Mammoths at the bottom. Table Queen and Fordhook, belonging to the *C. pepo* group, were moderately heavy, while the majority were comparatively thin. The species *C. moschata* contains no variety having a very heavy consistency.

The consistency of the product varied considerably with the age of the material. The very immature material, 10 to 20 days of age, had a very thin consistency when cooked or canned. Some varieties, like Delicious and Improved Hubbard, became much heavier in consistency from the 20- to the 40-day stage of maturity, while in other varieties this change was much less pronounced. Those varieties that increased greatly in total solids and acid-hydrolyzable polysaccharides increased correspondingly in heaviness of consistency. The consistency of the cooked products was heaviest at the 40- or 60-day stage of maturity. Those varieties with a heavy consistency at this stage generally became thinner in consistency during storage. The consistency of many varieties at the 40- and 60-day stages was considered too heavy for an ideal canned product. These varieties were greatly improved by storage for 30 to 60 days during which time much of the starch was changed to sugar.

When certain varieties, particularly those belonging to the species *Cucurbita maxima*, were canned by chopping the material into pieces ½ to 1 inch in diameter, packing them into cans, filling the interspaces with water, and then processing, the starch in the pieces cooked out into the water appeared white when cooled. The spaces between the pieces of material were solid or semisolid in character. This condition

made the product somewhat unattractive. It occurs sometimes in commercially canned pumpkin (*12*), in which case any space within the can not occupied by the pulped material becomes filled with the white starchy substance. These white spaces or patches have been called starch pockets or starch balls. There is a direct relation between the occurrence of these starch pockets, the consistency of the product, and the starch content of the material. Starch pockets are most likely to occur when the material is canned early in the season. In these tests it sometimes occurred even in such varieties as Large Cheese and New England Pie when canned very early. Starch pockets have been noted in Table Queen canned at 40 days of age but not in material that had been stored. Where this condition arises, it can almost always be improved by storing the pumpkins for 60 days or more. It may not occur even in varieties such as Delicious and Improved Hubbard after they have been stored for 60 days or more.

PALATABILITY OF IMMATURE MATERIALS

Most of the varieties may be used when immature, as is the custom with summer squash. At 20 to 30 days of age the fruits of some varieties of *Cucurbita maxima* might well be considered delicious. The varieties Tennessee Sweet Potato and Japanese Pie sometimes had an objectionable bitterness that made them less acceptable. Varieties with a low starch content appeared to have too much water or to lack substance. King of the Mammoths, Mammoth Chili, and Connecticut Field were varieties of this character. Delicious, Improved Hubbard, and Golden Hubbard were exceptionally good at 20 to 30 days of age.

NITRATES AND THE CORROSION OF CANS

A study of the canned material revealed a considerable difference among varieties in their action on the tin containers. Table Queen corroded cans very little, while Chicago Warted Hubbard often corroded them severely. Generally when nitrates were low or absent the cans showed little corrosion, but when they were high the action on the cans was very severe. Sometimes young material caused severe corrosion, whereas material stored for 30 to 60 days caused much less. Of course nitrates were not the only constituents favoring the corrosion of cans; oxygen in the container was also important.

DISCOLORATION

In these tests there was very little tendency for the material to darken during ordinary cooking or during canning. Many varieties were practically free of any objectionable darkening. This was probably due to the very low content of astringency or hydroxy-aromatic substances that react with iron. In some of the products a slightly dull appearance was noted, obviously due to the presence of chlorophyll in the fresh material.

SUMMARY

Thirty-six varieties of pumpkins and winter squashes, grown for 1 to 4 years, were analyzed at different stages of development and

after different periods of storage. Statistical analyses of the data indicated rather large differences in the extent to which many of the constituents varied, the sources of greatest variation being differences in age of the material and differences among varieties.

When calculated as percentage of the fresh weight the mean total-solids content decreased during the first 10 days of growth and then increased to the 40-day stage, after which there was a slow decline. The sugars increased during the developmental period and the first 60 days of storage. The acid-hydrolyzable polysaccharides increased rapidly to the 30- or 40-day stage and then almost as quickly decreased to the 90-day stage, after which the decline was slow. Although the changes were not very important, the acidity, astringency, and total nitrogen content decreased to the 10- or 20-day stage and then increased slowly to the end of the storage period. The nitrate nitrogen increased to the 20-day stage and then declined to the end of the storage period, the direction of the changes being the reverse of those in the total nitrogen.

When the results for each constituent for all stages of maturity in all years were averaged it was found that in general the varieties markedly high in sugar were also markedly high in acid-hydrolyzable polysaccharides; that those high in total solids were high in soluble solids and only moderately high in total nitrogen.

The varieties differed greatly, however, in the extent of the changes that occurred in the content of total solids, acid-hydrolyzable polysaccharides, and sugars. The differences in the acidity, astringency, and total nitrogen content of the varieties were of much less importance. There were comparatively small differences in most constituents of the fruits during the very early stages of development, but the differences often became pronounced as the fruit approached maturity. The varieties that were high in acid-hydrolyzable polysaccharides at the time of maturity generally became high in sugar during storage as a result of the conversion of starch into sugar. Among those varieties high in acid-hydrolyzable polysaccharides or sugar were Table Queen, Fordhook, Banana, Improved Hubbard, Golden Hubbard, and Sibley. Among those low in these constituents were Connecticut Field, King of the Mammoths, Large Cheese, Mammoth Whale, and Winter Luxury. *Cucurbita pepo* and *C. maxima* both contained some varieties that were high and others that were low in dry matter and polysaccharides, but *C. moschata* contained no varieties that were exceptionally high in these constituents. In a few varieties, such as Table Queen and Fordhook, nitrate nitrogen was almost absent after the fruits reached maximum size, whereas in other varieties, such as Marblehead and Kitchenette, appreciable amounts were present far into the storage period.

Differences in the flavor, consistency, and appearance of *Cucurbita* varieties were often very great and were directly related to differences in composition. Because of these differences some varieties that are excellent for one purpose may be only mediocre for another. The period when *Cucurbita* fruits have maximum value depends upon the variety and the particular use to be made of the fruits. Some varieties are excellent for certain culinary uses during the early stages of maturity and very poor for the same uses at later stages, while the reverse may be true of other varieties.

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REDUCTION IN PRODUCTIVITY OF MUSKRAT PELTS ON AN IOWA MARSH THROUGH DEPREDATIONS OF RED FOXES [1]

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INTRODUCTION

The muskrat (*Ondatra zibethicus*) (*2*)[2] constitutes a major economic resource in many parts of North America. Even in midwestern agricultural communities the income from the sale of pelts taken on superior muskrat marshes often has been equal to, or actually exceeded, monetary returns from farm crops and livestock raised on adjacent lands. In view of the decline of important fur bearers in recent years (*15*), it is not surprising that there should be a growing interest in muskrat management on a sounder production and cropping basis.

The muskrat is at times subject to conspicuous predation by certain types of flesh eaters, a fact which naturally arouses speculation as to how much damage may result and to what extent it may be averted through repressive measures against the predators. In Iowa, field researches begun in 1932 have demonstrated that the majority of muskrats known to have been killed by predatory enemies other than man or their own kind were victims of minks (*Mustela vison*), great horned owls (*Bubo virginianus*), and red foxes (*Vulpes regalis*). Analyses of mink and horned owl predation upon muskrat populations have already been reported (*6, pp. 191–192; 11; 13, pp. 792–793*); this paper records the data on red fox predation.

Although the foxes clearly have killed far fewer muskrats on the Iowa study areas than have the minks, fox predation, because of special aspects, may very conceivably have the greater economic significance. The distinction may be emphasized that intercompensatory or automatically adjusting trends in both reproductive and loss rates of the muskrats have served to nullify, in effect, most of the heavy as well as the light predation suffered (*9, p. 182; 10, pp. 166–167, 178–179; 11*). This means that as a rule the predation was borne by muskrats living under such disadvantages that they (or their numerical equivalents) were doomed anyway, if not from interspecific attack then from intraspecific, or from climatic emergencies or miscellaneous agencies. Fox predation, nevertheless, may not always be

regarded merely as substituting one loss for another, with inconsequential differences in end results; and dissociation of its noncompensatory from its intercompensatory phases is a main objective of this writing.

THE GENERAL PICTURE OF RED FOX PREDATION UPON MUSKRATS

Preying by red foxes upon muskrats is variously mentioned in North American literature, and it was quite to be expected that introduction of the muskrat into Europe should have been followed by reports of its utilization by *Vulpes vulpes* (*26, pp. 38–39; 27, pp. 195–196*). In the eastern part of the United States, muskrats seem to occur particularly in the spring diet of *V. fulva* (*1, 14, 16, 20, 25*), and their remains may be well represented about the dens where young foxes are kept (*14, pp. 20–21; 25, p. 19*). Smith (*25, p. 19*), reporting on muskrats in Maryland, stated that ". . . practically all trappers regard foxes as serious pests on a marsh. On June 27, 1934, a fox den from which the old fox had recently removed her cubs was visited. Among the food remains were found the skulls and other bones of 12 adult muskrats." Investigations of the food habits of *V. fulva* and *V. regalis* in the North-Central States brought out little evidence of muskrats being taken during "normal" years except to some extent in spring (*3, pp. 20–25; 4, pp. 192–193; 17; 24*).

In the spring of 1934, 38 muskrat carcasses comprised 1.33 percent of 2,848 prey items recorded from 200 Iowa red fox dens, as compared with 2 carcasses, or 0.2 percent, of 1,010 items from 113 dens in 1933 (*5, p. 56*), but the increased severity of the 1934 predation may be attributed in large measure to exposure of muskrats by the spring drought of that year. "Normal" spring vulnerability of these animals to predators, including foxes, is usually distinct from drought vulnerability and seems mainly to relate to transient, unmated male muskrats, which are often handicapped not only by unfamiliarity with the terrain and inferiority of the living quarters available to them but also by their own restlessness and the intolerance displayed toward them by members of population groups already in established residence (*9, pp. 176, 178; 11, pp. 833–841*). The habitual spring wanderers observed in Iowa have been a biological surplus and thus highly susceptible to predation—indeed a surplus that has tended to be dissipated so completely by early summer that the presence or absence of foxes, minks, or any other kind of wild predators seemingly has had scant influence on its fate. This has been true whether the surplus males were in good or poor physical condition, formidable fighters or scrawny individuals dying from wounds of intraspecific harassment.

Murie (*19, table 1*) found muskrat remains in 45, or 5.8 percent, of 768 red fox droppings gathered on the Edwin S. George Reserve in Michigan from January to August 1934. Most of the muskrat representations were listed for the spring months, but the same author (*pp. 36–37*) explains that the marshes had little or no water in them during the winter and he suggests that the ". . . muskrat under conditions more favorable to its mode of life than those existing on the George Reserve would probably be less vulnerable to fox attack." His description of surface activities of the muskrats is indicative of drought vulnerability comparable to that observed in Iowa under extreme conditions (*7, 11*).

The water levels of Round and Mud Lakes, two neighboring marshes north of Ruthven, in northwestern Iowa, were very low in November 1936. The muskrat population of 450-acre Round Lake averaged about 1 per acre; that of a 300-acre section of Mud Lake, about 1⅔ per acre (7). The proportion of wanderers was much higher on Mud than on Round Lake,[3] and both marshes were being hunted over by foxes. A single fox scat examined from Round Lake contained muskrat remains, whereas none of eight from Mud Lake did. Round Lake also had water over much more of its bottom than Mud Lake, hence more of it had been inaccessible to hunting foxes up to the late fall freezing; on Mud Lake, nearly 100 acres (about 40 of which were occupied by muskrats) had been dry enough to permit movement by foxes almost everywhere since midautumn. The Mud Lake foxes simply did not respond to the pronounced vulnerability of the muskrats.

Later in the winter, intensive studies of a drought crisis were carried on at Little Wall Lake, a 230-acre marsh south of Jewell, in central Iowa. The muskrat population entering the winter was but a remnant of what it had been months previously, and was calculated at about 40 individuals on about 100 acres still occupied (7). The marsh was entirely depopulated of muskrats by early February, and some data were acquired concerning the fate of about three-fourths of the animals that had entered the winter. The diets of local minks were made up to a substantial extent of muskrats, both of mink-killed victims and of carcasses of those dying from intraspecific wounds, hunger, and cold; fox "sign" was conspicuous on the dry marsh bed, but these predators did not appear to be especially interested in the muskrats. A fox was once noted (December 8) to have defecated on a muskrat that had died of muskrat-inflicted wounds, and, later (February 11), a fox scavenged upon a carcass of which parts had been dug out of the snow.

In the spring of 1937, Little Wall Lake was partially repopulated, 7 breeding females and associated males moving in from surrounding habitats to station themselves in about 90 acres of marsh habitable at the time. These were known to have given birth to 13 litters, totaling well over 100 young.[4] The marsh went dry in later summer and had only a remnant population by November, 16 individuals, as reported by trappers. Yet, aside from fragments of a muskrat that had probably died in the winter, no remains of muskrats were detected amid the prey debris littered about a series of marsh-edge trails and den holes used until late summer by a family of foxes.

Approximately two-fifths of the bottom of Cheever Lake, a 282-acre marsh area near Estherville in northwestern Iowa, was exposed by drought in the late fall of 1939. Muskrat densities on the wet marsh were high—1,680 being the figure arrived at on the basis of generally accurate data. In addition, about 85 animals were determined to be resident, though precariously situated, in dry habitats. Twenty-five carcasses, known to be chiefly those of victims of intraspecific strife and minks, were found between September 28 and November 19, and only 1 of 6 live specimens collected on shore for examination was

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[3] A deliberate effort to collect all wanderers on Round Lake yielded 4 live specimens, 1 dead, and gave indications of at least 1 escaping; the private fur catch from the completely exposed marsh bottom of Mud Lake was 42 pelts.

[4] Six of these litters were plainly complete when handled and averaged 8⅔ young.

without the strife wounds typically borne by drought-evicted wanderers at this time of year. Foxes hunted in accessible parts of the marsh, but no evidence was seen either of their scavenging upon carcasses of muskrats or hunting live ones. Conditions at Cheever Lake grew worse in 1940, and the last of the surface water disappeared on October 22, which resulted in severe mortality and final abandonment by nearly all of a remnant population estimated at about 200; foxes hunted even in the center of the lake bed but without leaving any observed evidence of killing or eating muskrats. In contrast, the minks responded conspicuously to the vulnerability of large numbers of muskrats brought about by the two autumnal drought crises (*11, pp. 859–865*).

From the foregoing review it is apparent that definite instances of red foxes preying heavily upon muskrats are not abundantly recorded [5]; hence a situation studied in 1940 at Wall Lake, a 935-acre marsh north of Blairsburg, in north-central Iowa, merits discussion in some detail. Preliminary references to predator-prey relationships on this marsh have been made in connection with the analysis of the mink predation (*11*).

METHODS OF INVESTIGATION

A regular program of field research on the muskrats of Wall Lake was begun by the senior author in May 1939. The standard investigative procedure was to try to locate all habitations of females with young [6] and thus to determine the foci of breeding "territories," to obtain data on size of litters and sexes and ages of individuals by techniques previously demonstrated (*8*), to mark the litter members by tagging (*12*) and toe clipping,[7] and, later, to obtain all possible data on behavior and mortality of the population until winter. When feasible, efforts were made to obtain for post-mortem examination an adequate number of specimens in November and December, the time of most fur trapping for Iowa muskrats. From these specimens, information on sex and age ratios, growth and developmental rates, numbers and comparative freshness of placental scars in the uteri of mature females, etc., were sought for correlation with the results of the preceding warm weather studies.

In 1940 the junior author began participation in the Wall Lake investigation, and thereafter the work was carried on jointly, each person assuming responsibility for data acquired for his own project. Hunting methods and related behavior of the foxes were deduced from tracks in the mud or peat bottom of the marsh, digging and trail "sign," and the appearance of prey remains. Scats of unquestionably fox origin were gathered, dated as closely as the evidence permitted, and finally examined in the laboratory with the aid of reference specimens.[8]

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[5] Pancoast (*22*) ascribes tremendous losses to red foxes on New Jersey muskrat marshes, but, while the writers do not doubt that much evidence of killing was observed, reductions in trapping yields to one-fourth or less of "normal" are not, in view of apparent variables, necessarily chargeable solely, or even largely, to fox predation.

[6] This task was simplified by the fact that fewer than 50 acres were then habitable for the species because of a low water stage; the resulting wide, flat shore margin, not being conducive to the maintenance of bank burrows, forced the females to keep their litters in lodges and nests where they could be easily reached by hand.

[7] ASHBROOK, F. G. MARKING WILD ANIMALS FOR IDENTIFICATION. U. S. Dept. Agr. Wildlife Res. and Mangt. Leaflet BS–57, 3 pp., illus. [Processed.]

It has long been recognized that neither fecal analysis nor any one method of studying food habits of foxes, when used alone, is free from disadvantages (*4, 23*), so the practice in this investigation has been to seek data by all practicable means and to try to derive conclusions from the total evidence available. Actually, the fox droppings did prove to be the best single source of information, but uneaten prey debris and other "sign" also contributed much of value; and the resulting ecological picture, though in many ways incomplete, can be said to reflect trends in predator-prey relationships.

There is no reason to believe that the presence of the investigators materially influenced the behavior either of the foxes or the muskrats, or of other wild animals importantly involved. The foxes showed a tendency to avoid parts of the dry marsh frequented by farm dogs but appeared tolerant of the research activities, which, of course, were conducted in ways intended to be least disturbing.

THE WALL LAKE CASE HISTORY

The Wall Lake muskrat population was calculated at 275 to 280 individuals on 34 acres of marsh occupied early in the winter of 1939–40. About 15 of the 34 acres constituted excellent wet marsh habitat, and here a population of over 200 was judged to have wintered with little loss. Elsewhere on the marsh, shallow water and continued dry weather afforded the muskrats little or no winter protection, and mortality from dogs, minks, hunger, and cold was pronounced. Survival figures of 18 to 22 out of 55 to 60 were arrived at for the inferior habitat, which would give a total survival of something over 200 muskrats for the marsh.

By the spring of 1940 there was sufficient water to bring the marsh level back to about where it had been in the late fall of 1939. Six tracts of fairly open water ranged in size from approximately 1 to 4 acres and were surrounded by dense stands of emergent vegetation, mainly bulrushes (*Scirpus* spp.), cattail (*Typha latifolia*), and reed (*Phragmites communis*).

The first careful inventory of "territories" in 1940 was made on May 30 and 31—at which time Iowa muskrats are usually in permanent summer residence—and the data procured then and later indicated an initial density of 41 to 46 females and possibly a somewhat larger number of males. Eleven of the females had their "territories" on 10 acres of the shallower parts of the marsh, and the 30 to 35 others had settled on the wetter 24 acres of the occupied area.

The breeding season was favorable for deep and shallow marsh muskrats alike throughout the first 3 weeks of June. Four of 30 mink scats that could be satisfactorily assigned to early May contained muskrat remains but these were probably all of the same victim; there were no representations of muskrat in 106 scats from late May and June. The maximum number of young muskrats present at any one time was estimated at between 500 and 700. Sixty-one young muskrats in 15 litters and a litter of 5 young minks were marked, but, of these only a single toe-clipped muskrat was subsequently recovered. [9]

[9] The writers' records show that 134, or 19.5 percent, of 686 Iowa muskrats yielded data after marking and that, under conditions favorable for study, it might be expected that about 30 percent of the individuals marked would be recovered.

By June 20, the marsh bottom of about half of the 34-acre occupied area was exposed except for puddles and water in the entrances of the lodges.   Friction between adult muskrats was observed on one of the drier tracts, and the situation was becoming critical for an increasing number of residents.   The species had not as yet suffered unusual mortality, however, although foxes were displaying interest in two sets of lodges.   This place, where the attention of the foxes was attracted by the shallow-water muskrats, was near the east-central shore, across the marsh from the apparent sites of the spring fox dens.   The foxes limited their explorations to areas of bottom firm enough to prevent their feet from sinking more than about an inch.   Minks frequented mainly the deeper marsh, where they were living almost entirely upon coots (*Fulica americana*) ; no muskrat remains were found in 30 of their scats deposited during the middle of June.

A local 3-inch rain on the night of June 22 had made little perceptible difference by June 24, and by June 26 the appearance of the marsh was much as it had been just before the rain.   Drought-exposed muskrats (calculated at 30 to 50 individuals, in the ratio of about 3 young to each adult) were foraging in the vegetation near the lodges and extending their diggings.   Foxes, while including cottontail rabbit (*Sylvilagus floridanus*) and miscellaneous beetles in their diet, were now taking full advantage of the muskrats, all of 6 scats containing remains of young animals 6 to 10 weeks of age.

During July the drought increased in severity and dried out the habitats of between 50 and 200 muskrats.   By the early part of the month, the foxes, plainly hunting as a family group and systematically working muskrat feeding grounds in the vicinity of newly exposed lodges, had caught nearly all the young muskrats in the tract that first went dry.   They next worked southward in the marsh, keeping close to the edge of the receding water (often going so close as to sink 2 or 3 inches in the mud), and by the end of the month had caught practically all except the adult muskrats on at least 20 acres of marsh. Three carcasses of adult muskrats were located but these were more likely victims of intraspecific strife than of predation; fragmentary carcasses of 6 young between 5 weeks and 2 months of age, probably or certainly killed by foxes, were examined.   Forty-five of 47 fox scats contained muskrat remains, mostly of animals between 2 and 3 months old.   Minks seemingly avoided the places heavily hunted by the foxes and were not known to have killed muskrats in any place where surface water remained in the lodge entrances.   The minks did hunt in a dry corner of the marsh that, as yet, had not been exploited by the foxes.   Four of eight mink scats contained remains of about the same age classes of young muskrats as the fox prey.

Fox diets during July reflected highly opportunistic hunting.   In addition to the muskrats, other inhabitants of marshlands were taken, especially beetles.   Of 47 fox scats examined, 19 contained avian remains, those recognized being mainly of coot and duck (Anatidae). Garter snakes (*Thamnophis*) were abundant on the dry marsh bottom, and were fed upon, as were crayfishes (*Cambarus*).   A huge snapping turtle (*Chelydra serpentina*) had been dug out of the mud and most of its flesh had been eaten; turtle remains were found in 1

scat. Single representations of cottontail and shrew (*Sorex cinereus*) and 2 of young meadow mouse (*Microtus*) were present in the 47 fecal samples.

In late July, the fox family (the "sign" indicated a group of four animals) was centering its activity in the south-central part of the marsh. Hunting techniques, as revealed by tracks, seemed to entail a definite system of maneuvers, some foxes stationing themselves beside the trails between muskrat lodges and feeding grounds while others circled about. It is probable that the effectiveness of the system resulted not so much from genuine teamwork as from the astuteness of old foxes in waiting near places where alarmed young muskrats would be apt to run. No evidence of digging into muskrat-occupied lodges or burrows was disclosed, although a nest of incompletely hatched snapping turtle eggs was dug out of the top of an old lodge.

By the beginning of August the water level was so low that a muskrat population of possibly 250, with a ratio of about 2 young to each adult, had been exposed. Only 1 carcass of a fox victim (a young animal of about 3 months) was found, but 18 of 20 fox scats deposited up to the middle of the month contained remains of muskrats, chiefly of young of 2 months to subadults of 3½ to 4 months. There was still no evidence of foxes successfully catching adult muskrats, though the minks were killing some in the long-exposed tracts that the foxes had virtually depopulated of young and then abandoned. Muskrat representations (including 3 of adults) were listed for 4 of 7 mink scats picked up in such places, and 20 scats from wetter habitats had remains of young or subadults in 3.

By August 6 rains filled the deeper part of the marsh to a depth of several inches and by mid-August the latter habitat (which not only had the highest density of muskrats but also had been the last to go dry) was well reflooded. With this reflooding, the foxes gradually discontinued their lowland hunting and spent much of their time along the southwest shore of the marsh and on the adjoining cultivated land. A trail of about 200 yards extending through dense vegetation from shore to the west fringe of the wet area showed heavy passage, and there was evidence of foxes using old lodges as beds. Although one muskrat was known to have spent some time in a cornfield that was the principal headquarters of the foxes in late August, there were probably no more than a dozen vulnerably situated muskrats about the entire marsh at this time, and these seemed to be transients, both in the marsh and on high ground. Muskrat representations were found in 2 of 20 mink scats and in 3 of 11 fox scats deposited from late August to the middle of September, and a shift in dietary trends of the foxes from muskrats to rabbits, mice, and birds became apparent.

After the middle of September, the foxes moved northward into the vicinity of what were believed to have been their former breeding dens, thus completing during the summer season a clockwise circuit with a radius of about a half mile; the total area hunted over during this period was certainly not much larger than a square mile.

By September 25 the marsh was again going dry, and by the middle of October the bottom was fully exposed. Muskrat remains were

found in 4 of 11 mink scats but in none of 6 fox scats. The foxes no longer spent much time in the marsh, despite the fact that a population of over 200 muskrats in ratios of 1.9 young to each adult and 3.1 young to each adult female lived vulnerably in habitats with which the foxes were familiar.

Trapping by the public in November practically annihilated the muskrats, the catch being 197. Of these, the carcasses of 105 were examined. There was evidence of a few trap cripples wandering over the frozen marsh after the trapping ceased, but no muskrat representations were found in 145 fox scats deposited from October 1940 to early March 1941.

The marsh was restocked naturally with muskrats to a limited extent during the 1941 spring dispersal of that species from stream habitats in which winter survival had been better, and the summer population of adults was ascertained to be 5 pairs (or equivalent) and 1 unmated or nonbreeding female. During the early stages of the dispersal in the first half of March, a considerable number doubtless moved about the countryside. On March 12, a freshly killed, maturing male muskrat was found at a den at which newly born fox pups were kept, and 1 scat passed between March 16 and March 20 was of muskrat remains; since the first carcass had been taken to the laboratory before the foxes had eaten of it, the fecal representation was of a different victim. No other muskrat remains were found in the rest of a collection of 194 scats (51 of these were pup scats) deposited from the middle of March through April. The foxes subsisted mainly through the fall to spring period of 1940–41 on rabbits, mice, and birds.

The fox pups were judged to be 6 or 7 weeks old by May and, by the middle of the month, were penetrating about 100 feet into the vegetation at the west-central side of the marsh. By this time, several sets of more or less neighboring holes had been used as den headquarters; by June 10, the fox family (there were at least four young) seemed to be ranging free from the dens and, by the end of the month, had definitely abandoned them.

August 1941 brought another drought to the Wall Lake muskrats, but they were relieved by rains after about 3 weeks of exposure. No tracks of foxes were seen in the dry central parts of the marsh, however, and no muskrat remains were found in 555 scats deposited from May through August.

No notable mortality was suffered by the muskrats in the fall until the local population was once more virtually annihilated by public trapping. The sex and age ratios of the carcasses obtained from trappers in December suggested a rate of increase of only 4.6 young per breeding female, although the average of the season's placental scars was about 30—which represents a typically severe reduction in young for the length of time that they were exposed by drought.

No sign of family association of foxes was seen after early September and, by early October, the evidence of foxes noticed on the area was northeast of the marsh. Thirty-three scats collected from September 1941 to January 1942 showed the usual diet of upland vertebrates.

ANALYSIS OF THE FOX PREDATION UPON THE WALL LAKE MUSKRATS

From previous and contemporaneous investigations, one would expect a great deal of flexibility in general predator-prey relationships in the north-central part of the United States (*11; 13*). Most predatory higher vertebrates studied in this region have shown slight evidence of obligatory dependence upon any one or any group of prey species, even of species usually classed as staple prey. It is, therefore, unlikely that shortage of a prey species often served to force a predator into any particular diet, although a versatile predator no longer able to exploit its customary prey would naturally turn to one or more of the other species remaining available.

Availability of prey, relative or absolute, appears to be of outstanding importance in determining the food habits of the common raptorial birds and carnivores, but responses to changing availability may also be conditioned by other factors. Lack of responsiveness of one predator to increased availability of a given prey species may or may not be accompanied by greater response on the part of other predators. The point to be recognized is that, irrespective of the fundamentally mechanical aspects of predation, the role of chance and the differences in adaptability, experience, hunting routines, and predilections of the predators may be sufficiently influential to make attempts at very detailed cause-and-effect appraisals impractical, if not downright unreliable. And a participant capable of as much individuality as the red fox adds more than usual variability to the predator-prey equation with which we are here concerned.

The factors governing availability of prey to general-feeding predators may be exceedingly complex in analysis, but fall into a number of fairly clearly-defined categories. Broadly, the more abundant a species, the greater is the likelihood of its being preyed upon, especially in its immature stages (*18*). Among vertebrate species showing "territorial" intolerance (*21*), thresholds of security often exist, and populations in excess of these thresholds may be notably vulnerable to predation (*11, 13*). Anything, in short, that promotes disharmony or instability in vertebrate populations increases vulnerability to predatory enemies.

The Wall Lake case history depicts many responses of foxes to changes in the availability of foods. Rabbits and mice were the dietary staples; for one reason or another they almost always continued to be available at all seasons to avian and mammalian predators generally, as well as to the foxes. When the sudden Armistice Day blizzard of 1940 killed immense numbers of late-moving, red-winged blackbirds (*Agelaius phoeniceus*), the foxes immediately responded to this source of convenient and acceptable food. An apparent overpopulation of pheasants (*Phasianus colchicus torquatus*) massed near a cornfield in late November and December 1940 was not overlooked, nor were field-ranging chickens from farmyards in the summer of 1941. During the drought of 1940, as indicated, even the marsh-dwelling turtles suffered predation from foxes.

The predation upon the drought-exposed muskrats, with some differences, was much according to previously observed patterns. The

differences, nevertheless, were in many ways significant because of the specialized and effective hunting techniques of the foxes and the combination of variables arising from weather and unknown factors.

Presumably, the foxes were going to the water's edge to drink in June 1940 when they were attracted by a group of land-active, insecure muskrats. It might not be demonstrably correct to say that the latter were prey truly preferred by the foxes above all others but, at any rate, they became objects of unquestionably deliberate search. Furthermore, the muskrats of the shallow-water zones had suffered, through the enterprise of the foxes in 1940, nearly complete loss of their season's young, days or even weeks ahead of any ordinary schedule of drought mortality.

Had it not been for the fox depredations, the breaking of the drought by the August rains would either have averted the greater part of the midsummer losses or would have resulted in something of a postponement until the recurrence of the drought in the fall. It is conceded that many of the muskrats eliminated in the summer by the foxes would surely have died anyway from mink predation, intraspecific strife, etc., during the fall crisis, but, taking into consideration as well as we can the intercompensations indicated by the data, it may be concluded that an actual uncompensated lowering in productivity of between 50 and 100 muskrats must be charged to the foxes (*11*, *p. 881*).

This would mean, on the basis of the average of $1.21 received by Iowa trappers for 1940–41 muskrat pelts,[10] a net loss possibly exceeding $100.00 in trappers' income from the marsh—possibly a reduction of about 25 percent in the value of the fur returns. It is not believed that the predation by foxes or other wild animals had depletive influence on the breeding stock of the following spring, for, under the circumstances, human exploitation would probably have been as annihilative if there had been no "natural" predators in the vicinity.[11]

In 1941, the foxes, which there is some reason to believe included the original pair of adults, caught about as many of the spring-dispersing muskrats as they could be expected to encounter at random; on the other hand, they apparently did not happen to hunt in those places where the muskrats were again exposed by drought, so the predation sequences of 1940 were not repeated.

SUMMARY

In the summer of 1940 a study was made of an exceptionally drastic predation by red foxes upon the drought-exposed muskrat population of Wall Lake, a marsh in north-central Iowa. The severity of the crises confronting the muskrats doubtless would have resulted in heavy losses in the absence of the foxes, but to the specialized and effective hunting techniques used by the latter may be ascribed a reduction in muskrat numbers that seemed to a considerable extent noncompensatory. A possible net decrease through the fox depredations of about 25 percent of the trappers' income from the marsh is indicated by the data obtained.

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[10] IOWA STATE CONSERVATION COMMISSION. NUMBER AND VALUE OF FURS TAKEN IN IOWA, 1940–41 SEASON. Iowa State Conserv. Comm. 1941. [Processed.]
[11] Readers interested in the problem of overtrapping as it relates to muskrat management may consult an earlier paper (*9*).

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RANGE OF SOIL-MOISTURE PERCENTAGES THROUGH WHICH PLANTS UNDERGO PERMANENT WILTING IN SOME SOILS FROM SEMIARID IRRIGATED AREAS [1]

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INTRODUCTION

It has long been known that the wilting coefficient, or wilting point, of a soil does not represent the lower limit of soil moisture available to plants but rather the approximate lower limit available for growth (*6*).[3] Some writers, however, have used the term rather loosely to refer to the percentage of nonavailable moisture, possibly on the assumption that the amount of moisture available at moisture percentages below the wilting point is so small as to be of no practical significance.

Alway (*1*), Batchelor and Reed (*3*), and others have reported finding soil under deep-rooted trees or shrubs at moisture percentages well below the wilting coefficient and in some cases at about the hygroscopic coefficient. These writers pointed out the significance of the moisture below the wilting coefficient in the maintenance of life in these plants during periods of prolonged drought, and Batchelor and Reed proposed that, since the wilting coefficient does not represent the lower limit of available moisture, the hygroscopic coefficient be used as the reference value for expressing the relative wetness of a soil as related to plant behavior. While the hygroscopic coefficient, in the sense in which this term was employed, is no longer in general use as a soil-moisture constant, Batchelor and Reed's contention that a soil-moisture constant, approximately equivalent to the nonavailable soil-moisture percentage, is needed, is nevertheless a pertinent one.

In recent investigations of the response of citrus trees to various soil-moisture conditions (*10*), it was found that the water deficit of trees in the field was related not only to the proportion of soil in the root zone that was reduced to the first permanent wilting point, but also to the extent to which the moisture content of the drier parts of the soil in the root zone was reduced into the wilting range.

The wilting range is the range in soil-moisture percentages in which plants undergo progressive permanent, or irreversible, wilting, from

wilting of the oldest leaves to complete wilting of all the leaves. The lower end of this range has been termed the "ultimate wilting point." In this paper wilting is called permanent if turgor is not regained by the uninjured leaves when the plant is kept in an approximately saturated atmosphere in a dark humid chamber for 14 to 16 hours.

The terms "wilting range" and "ultimate wilting point" were introduced by Taylor, et al. (*17*) to describe the soil-moisture conditions in plots of native California shrubs, where they found that the soil-moisture content, to a depth of 6 feet or more, was appreciably below the first permanent wilting point at the end of the dry season. They defined the wilting range as the range in moisture content of the soil between the wilting coefficient and the ultimate wilting point, and the ultimate wilting point as the moisture content at which all the leaves remain completely wilted in a humid atmosphere. That is, the ultimate wilting point represents approximately the lower limit of the range of soil-moisture percentages in which plants are able to maintain life, though at this stage many of the leaves and probably some of the roots are dead. Even at the ultimate wilting point a small amount of soil moisture is, of course, still available to living tissue, and it might be supposed that the logical end point in determining the available moisture held by a soil would be the soil-moisture percentage at which the process of dying had just been completed.

Such determinations would, however, be time consuming, and the time when death was complete would be uncertain; furthermore the percentage of moisture at the death point is numerically practically the same as that at the ultimate wilting point, though, of course, at such low moisture percentages a small change in moisture represents a relatively large change in the vapor pressure or moisture tension of the soil.

Since the first permanent wilting point, or wilting coefficient, corresponds to approximately the soil-moisture percentage at which elongation ceases, and since the ultimate wilting point represents practically the lower limit of soil moisture that can be utilized by the plant in maintaining life, it would seem that in studies dealing primarily with the response of plants to soil-moisture deficiency these two reference values should provide adequate bases for comparison with most soil-moisture conditions encountered.

The wilting range, as here defined, has been reported for only a few soils (*10*, *17*). Since more general information was needed in connection with irrigation experiments, it seemed desirable to determine the wilting range of a large number of soil types varying widely in texture and other characteristics.

METHODS

Samples of about 80 soils, representing about 50 soil types as shown on soil survey maps of several areas of southern California, were collected for the study. With few exceptions the soil samples were taken from the top foot of soil, and most of them were from cultivated, irrigated orchards or fields. A few samples were from uncultivated desert lands or brushlands. The samples were air-dried and screened through a 2-mm. round-hole screen.

Moisture-equivalent determinations were carried out with standard apparatus, and the precautions recommended by Veihmeyer et al. (*19*) were observed. Four samples of each soil were run, and the average was taken as the moisture equivalent. In a few instances in which the four samples showed unsatisfactory agreement the determinations were repeated.

The wilting range determinations were made during the period from September 1940 to May 1941 at Pomona, Calif. The procedure for determining the wilting range is simple, but certain precautions were found to be necessary in order to obtain reliable results. The method employed proved to be convenient, economical of time, and reasonably reliable. Ten cultures with each soil sample, 5 for the determination of first permanent wilting and 5 for the determination of ultimate wilting, were run. The plant containers used were pint, compression-top cans, with ¾-inch holes punched in the centers of the covers. Four or five hundred grams of soil, depending upon the volume weight, was weighed into each can. Weighings were made on a spring food balance of 1-kg. capacity, and the groups of 10 cultures were placed on flat trays on the greenhouse bench. Russian Giant sunflower (*Helianthus annuus* L.) seedlings which had just shed the seed coats were transferred from flats of sand to the soil culture, 1 plant to a can.

It was found that frequently plant growth was poor unless care was taken to maintain ample soil pore space in the cultures. Jarring the cans after filling them caused appreciable settling of both dry and wet soil; so when the seedlings were transplanted to the cans, all the soil was poured out of the culture can into another container and then poured back into the can through a funnel while the plant was centered in the can by means of a small planting guide shown in figure 1, *A*. The roots of the seedlings were dipped into a soil suspension just before they were planted. This coating of the roots with a thin layer of fine soil at planting hastened recovery from transplanting. This improvement in recovery must have resulted either from protecting the absorbing surfaces from momentary drying or from improving contact between soil and roots, since it was found that regardless of treatment, the old root tips did not resume growth after transplanting but that new root growth arose from lateral root initials, which appeared about 2 days after transplanting.

After the seedlings were planted, the surface of the soil in the can was covered by a layer of absorbent cotton and the lid was fitted in place; then the culture was placed on the spring balance, and the weight of water required to wet the cotton, plus the calculated amount required to raise the moisture content of the soil to field capacity, was added from an overhead supply bottle. The cotton was placed on the soil surface to protect it from being puddled when water was added and to encourage the development of roots in the surface layer of soil. To loams and clays, nitrogen (50 to 100 parts per million of soil) was added in the water at planting and a small amount of monopotassium phosphate (KH_2PO_4) was mixed with the dry soil before planting. The sands were watered at planting with a complete nutrient solution. The first set of cultures run were fertilized with nitrogen only, and determinations on some of these soils had to be repeated because the lower leaves were severely affected by symptoms of malnutrition and failed to show normal recovery after temporary
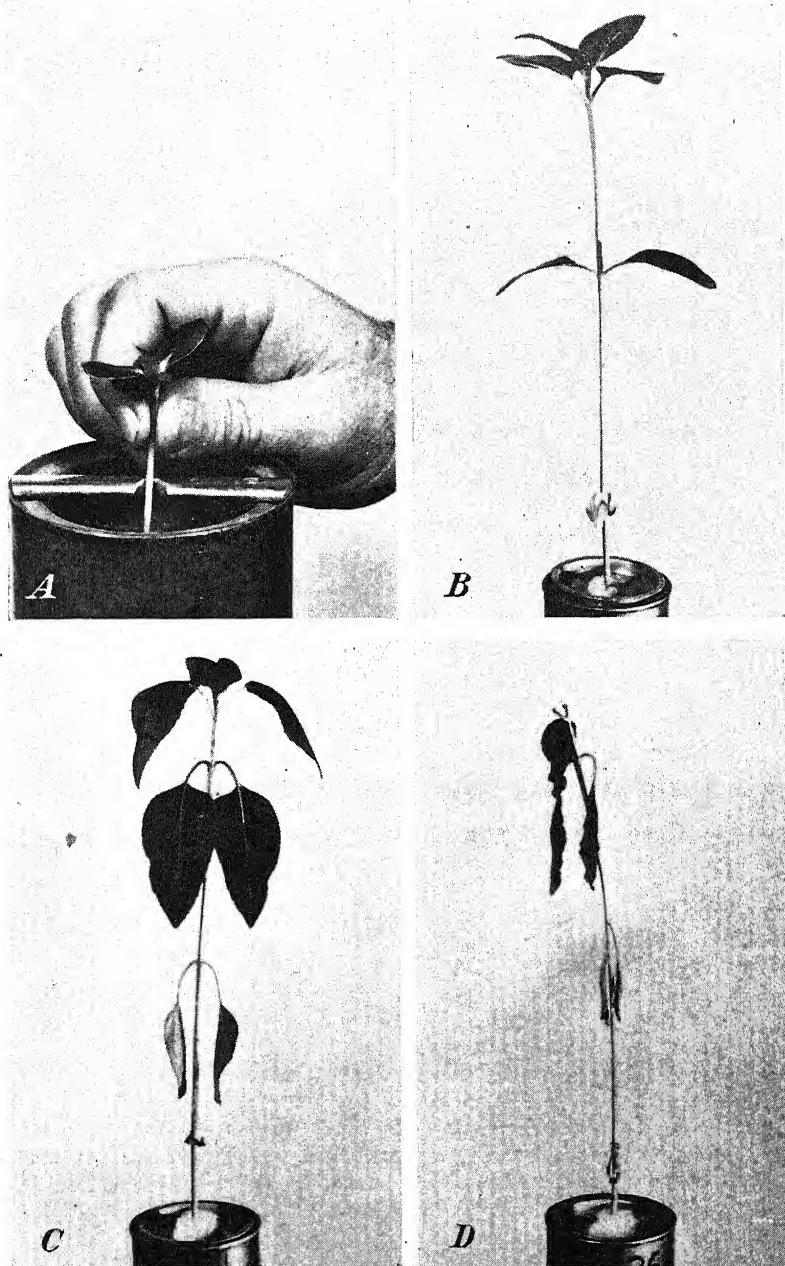
FIGURE 1.—*A*, Transplanting seedling to culture can; *B*, plant that had recovered turgor in the humid chamber after being wilted; *C*, plant at first permanent wilting point; *D*, plant at ultimate wilting point.

wilting. For a day after planting, the cultures were protected by a cloth shade, and then they were grown in full sun until the third pair of leaves had reached almost full size. Whenever some of the plants of a group in the same soil showed temporary wilting, water was added in sufficient quantity to bring the soil up to the estimated weight at field capacity, allowance being made for the increasing weight of the plants in estimating the quantity of water needed. When the third pair of leaves was almost fully developed, the soil was wetted to the estimated field capacity, the lid opening around the stem was closed with cotton, and watering was discontinued. As soon as cultures showed temporary wilting of the first pairs of leaves, they were placed under a cloth shelter, where they usually recovered turgor unless the leaves had been injured by fungus infection or malnutrition.

The lowest pair of leaves of a plant inadvertently left in full sun until several pairs were badly wilted usually showed injury and failed to recover turgor. Such plants could not be used for determining the first permanent wilting point.

When the lowest one or two pairs of leaves of cultures used for determining the first permanent wilting point wilted under the cloth shelter, the cultures were transferred to a dark humid chamber. High humidity was insured by exposing a large water surface in the chamber; in addition, just before the chamber was closed, the air was filled with a fine mist from a hand sprayer. As the plants recovered turgor, they were returned to the cloth shelter. This procedure of transferring the cultures back and forth from cloth shelter to humid chamber was continued until the basal pair of true leaves failed to recover after being in the humid chamber overnight. At this stage the tips of the second pair of leaves usually showed partial loss of turgor and drooped slightly. During the course of this process the time required under the cloth shelter before temporary wilting was induced gradually shortened until finally only a few minutes were required, and conversely the time required for recovery increased until finally recovery failed to occur within the overnight period of 14 to 16 hours. Since large numbers of plants can be transferred at one time, the labor involved in handling cultures in this manner is not prohibitive. The appearance of typical plants after recovery from temporary wilting and at the first permanent wilting point is shown in figure 1, *B* and *C*.

After the cultures used for determinations of the first permanent wilting point were watered for the last time, measurements of the stems were made early each morning with a ruler fitted with a sliding sidearm which could be brought into firm contact with the terminal growing point. Stem length was measured with an error of only about 1 mm. It was found that cessation of stem elongation coincides approximately with first permanent wilting as judged by the condition of the basal pair of leaves of normal plants. There was, however, some variability; some vigorously vegetative plants continued to elongate several millimeters a day for 1 or 2 days after the lower leaves were judged to be flaccid at the end of the 14- to 16-hour period in the humid chamber. Since, however, this rate of growth was very low as compared with the normal rate when the cultures were first sealed, approximate cessation of stem elongation was used as a secondary

criterion of first permanent wilting and was especially useful with plants that had suffered some injury of the lower leaves but were not so badly injured as to be considered unsuitable for use.

The cultures used for determining the ultimate wilting point were left under the cloth shelter until the apical leaves were badly wilted. Unless the apical leaves are severely wilted, it is difficult to tell whether they show recovery in the humid chamber. At this stage the stems were frequently flattened and distorted and the older leaves were dead. When placed in the humid chamber they never showed more than very slight signs of recovery of even the apical leaves. The appearance of a typical plant at the ultimate wilting point is shown in figure 1, *D*. At the ultimate wilting point the terminal one or two pairs of leaves, the terminal growing point, and at least some of the axillary buds are alive and the plant will resume growth if the soil is wetted.

When the plants were judged to be at the first permanent wilting point or at the ultimate wilting point, they were pulled out of the soil so that the largest roots were removed. The top layer (about one-half inch) of soil, in which root concentration was usually low, was removed and discarded. The soil from the upper and lower halves of the culture cans was sampled separately. The moisture content, determined as loss under drying at 105° C., is expressed as percentage of dry weight. A comparison of the values for the two samples served as a convenient check on possible errors in manipulations or calculations, but the average of the two was taken as the moisture content of the culture, and the average of the five cultures was taken as the moisture percentage at the first permanent or at the ultimate wilting point. The moisture content of the soil from the lower halves of the cultures was usually 0.1 to 0.3 percent higher than that from the upper halves. This difference may have resulted from differences in root concentration, soil temperature, salt concentration, or loss by evaporation, but the cause was not determined.

The widest variations between the moisture percentages of the five cultures of a sample at the first permanent wilting point or at the ultimate wilting point ranged from about 0.3 in sands to as much as 2.1 in the heaviest clay. These variations resulted in part from variations in judgment of the several workers who handled the cultures and in part from unavoidable variations in severity of wilting before the plants were placed in the humid chamber for the last time. Slight variation may be expected also as a result of variability in the amount of mechanical tissue in plants grown under different conditions of nutrition, water supply, temperature, and other environmental factors.

In the literature wilting-point values are sometimes reported to hundredths of a percent of moisture, and usually the probable or standard errors reported are small. Perhaps such data imply that the methods used in wilting-point determinations lend themselves to greater precision and reproducibility than may actually be the case. It is to be expected that variations between individual cultures of one soil run at the same time by one worker will be small, but over a period of several months or years an individual worker's notion of what the plant looks like at some selected stage of wilting, as first permanent wilting or ultimate wilting, may vary considerably unless some well-

defined criteria are established for judging when the selected stage has been reached, and it is almost certain that the stage of wilting classed as permanent wilt has varied widely as judged by different workers in widely separated laboratories.

To obtain an indication of the variability which might be expected in wilting points run on the same sample of soil at different times, cultures from a large sample of clay loam were run through the usual procedure in the fall, winter, and spring along with other soils, so that only the usual care would be given them. The average values obtained for first permanent wilting point in fall, winter, and spring were, respectively, 19.9, 20.2, 20.3; and for ultimate wilting point, 17.3, 16.9, 17.0. The difference between the extremes in each case was 0.4 percent. That rather consistent results may be obtained in routine wilting-point determinations by the procedure described was demonstrated by the results obtained on samples from 12 field plots on a relatively uniform clay loam. Each sample was an unscreened composite of eight 4-inch auger borings distributed uniformly over each of the plots, which were about 160 feet long and 24 feet wide. The first permanent wilting points of the 12 samples ranged from 20.3 to 21.9, and the moisture equivalent ranged from 32.7 to 36.1. In routine field work the average value of first wilting point or ultimate wilting point for these plots might be used for any one of the plots without serious error.

The precautions found to be helpful in obtaining reliable first permanent wilting points may be summarized as follows:

(1) Good root growth and distribution should be obtained by maintaining ample pore spaces in the soil and avoiding wetting the soil far above field capacity.

(2) Vigorous and healthy plants should be obtained by maintaining favorable nutrition, preventing infection by fungi, and avoiding overheating of the soil in culture cans exposed to full sunlight.

(3) When the plants wilt, they should be placed in the humid chamber before excessive desiccation causes injury to the lower leaves.

(4) Daily measurements of stem elongation should be made as a supplementary indication of first permanent wilting. Basal leaves that have become senescent or have been injured, even though not showing obvious signs of injury, may fail to recover when the plant is still receiving enough water to cause appreciable growth. Plants elongating at a rate of more than about 10 or 15 percent of the rate maintained during the first 1 or 2 days after the final watering should probably not be considered as permanently wilted.

RESULTS

PLANT RESPONSE TO DECREASING SOIL MOISTURE

In general, the daily rate of stem elongation decreased soon after the cultures were sealed and watering was discontinued, but no definite conclusions as to the relation between growth rate and soil moisture above the wilting range can be made, because as soon as temporary wilting occurred the plants were placed in partial shade and part of the time in a dark humid chamber. It is clear, however, that even under these conditions elongation had almost or entirely ceased at the first permanent wilting point. Growth rate curves typical of the plants grown in soils of different textures ranging from sand to clay

are shown in figure 2. Measurements of stem elongation were also made on a number of plants used in determining the ultimate wilting point. These plants were placed under the cloth shelter when they first became temporarily wilted, but were not placed in the humid chamber until all the leaves were severely wilted. Elongation had approximately ceased when the basal leaves remained wilted overnight, and the stems, because of water loss, decreased in length before the ultimate wilting point was reached.
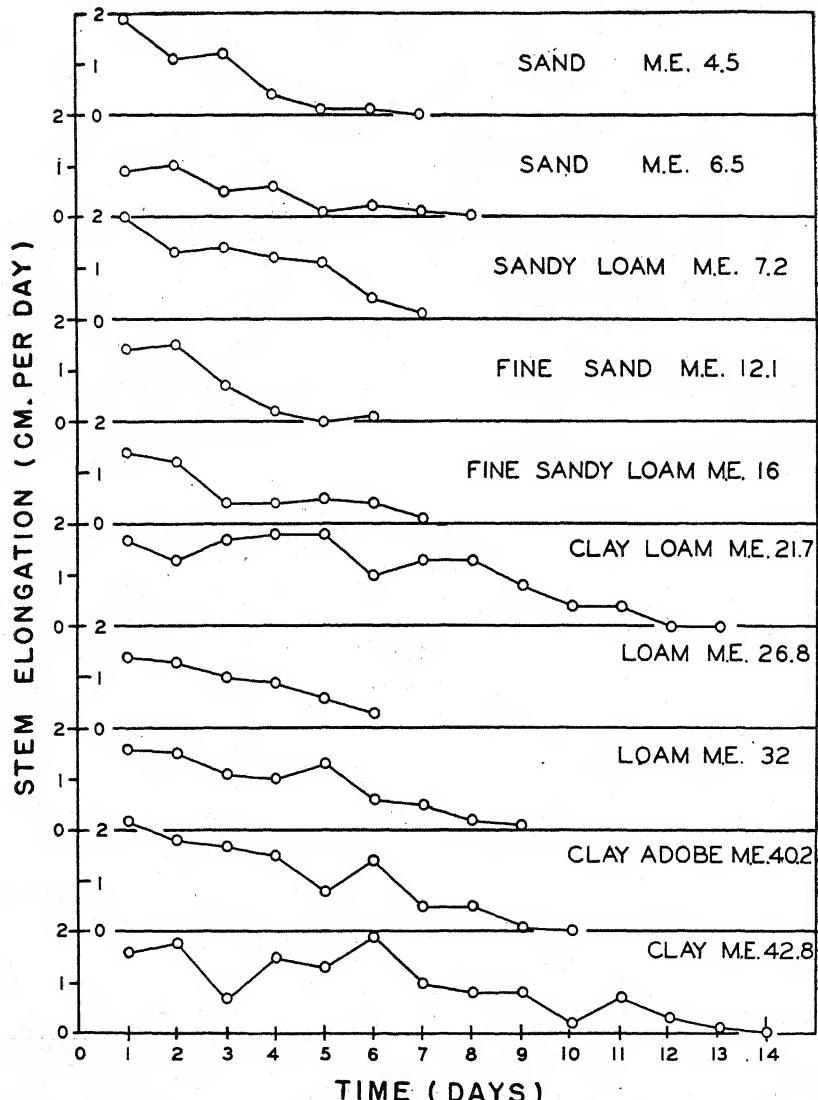
FIGURE 2.—Daily rate of stem elongation of sunflower plants in different soils accompanying decrease in soil moisture from field capacity (when growth measurements were begun) to first permanent wilting point (when last measurements were taken). Moisture equivalents (M. E.) given.

An attempt to estimate the changes in force exerted on water at the root surface during the progress of the decrease in moisture content of the soil from about the field capacity to the ultimate wilting point was made by determining the freezing point of samples of sap pressed from the plant tops and then calculating the equivalent osmotic pressure corresponding to the freezing-point depression. The plant tissue was kept frozen until just before the sap was extracted in a hydraulic press. Samples were taken from plants under two sets of conditions. One set of cultures was wetted to above field capacity and sampled at intervals as the soil dried out. The forces acting on the water in plant and soil were not brought to equilibrium by placing the cultures in a humid chamber; but the force gradient between plant and soil was comparable with that in the field at sunrise, since the
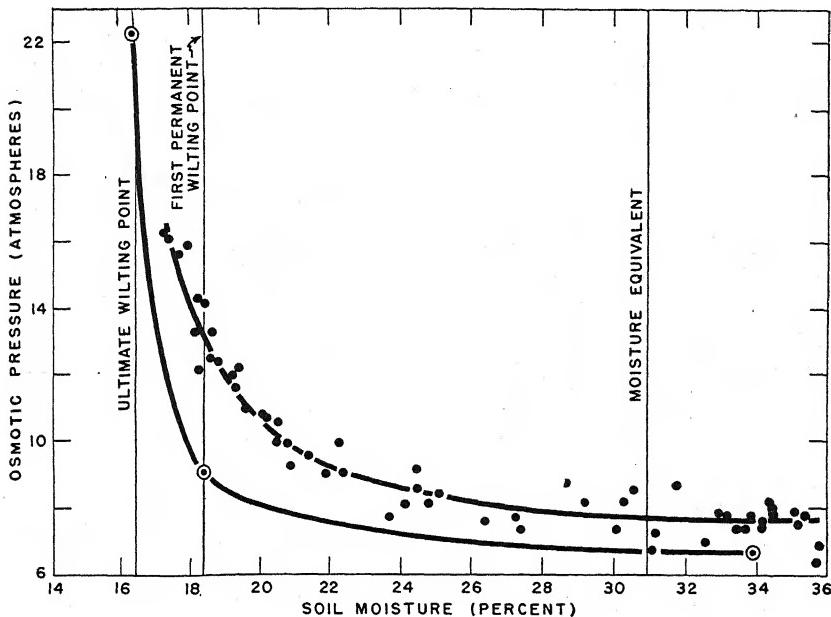
FIGURE 3.—Osmotic pressure of expressed sap from sunflower plants in soil at different soil-moisture percentages. Upper curve: Plants continuously in dry air; sampled at end of the dark period. Lower curve: Plants sampled after 16 to 24 hours in humid chamber.

plants were placed in a room overnight where the relative humidity was only slightly less than that of the outside air and then were sampled after a night of relatively low transpiration. The calculated osmotic pressure of samples of sap from these cultures is shown by the upper curve in figure 3.

These data (fig. 3, upper curve) show that as the moisture content of the soil decreased from about 24 percent to the lowest value reached, about 17 percent, there was a definite increase in the osmotic pressure of sap. Moisture determinations made on plant tops from these cultures showed that with decreasing soil moisture there was a concurrent decrease in moisture content of tissue. It is apparent that there

is a progressive change in the slope of the curve as the moisture content of the soil decreases below about 24 percent, but there is not a sharply defined break at 18.4 percent, the first permanent wilting point. At soil-moisture percentages several percent above the first permanent wilting point, the basal leaves of these plants failed to recover turgor during the night. It is clear that these plants, grown under atmospheric conditions resembling those in the field but with the roots rather uniformly distributed throughout a small mass of soil, were subjected to progressively increasing water deficit from a soil-moisture percentage about halfway between the moisture equivalent and the first permanent wilting point to a value below the latter.

The second set of cultures was sampled at only 3 different soil-moisture percentages. This set of cultures was divided into 3 groups. One group of 3 cultures was sampled at a soil-moisture percentage slightly above field capacity; another, of 17 cultures, at the first permanent wilting point; and a third, of 18 cultures, at the ultimate wilting point. The force gradient from plant to soil was presumably reduced to a very low value by placing these cultures in approximately saturated air for a period of 16 to 24 hours just before sampling. The cultures used for first permanent wilt were in the humid chamber most of the time for several days prior to sampling as well as for the last 16 to 24 hours. The cultures sampled at field capacity were presumably at full turgor.

The calculated osmotic pressures of the sap from plants at full turgor ranged from 6.4 to 6.8 atmospheres and averaged 6.6 atmospheres; at the first permanent wilting point the osmotic pressures ranged from 8.4 to 10.3 and averaged 9.1 atmospheres; and at the ultimate wilting point they ranged from 18.8 to 24.3 and averaged 22.2 atmospheres. The average values at full turgor, at the first permanent wilting point, and at the ultimate wilting point are shown as an assumed curve (lower curve) in figure 3.

The value of freezing-point determinations on expressed sap as an indirect measure of the forces acting on water at the root surface may be questioned, since the sap was extracted from the plant tops rather than from the roots and the wall pressure of the cells was not known. In spite of these apparent shortcomings, however, it seems probable that the method is fairly reliable. Water is translocated from one part of the plant to another so readily that apparently little difference in diffusion-pressure deficit exists in different parts of the plant unless transpiration is quite active (*2, 9*). Therefore, there was probably very little difference between the diffusion-pressure deficits of tops and roots of the plants that were kept in the humid chamber most of the time for several days before sampling.

Under most conditions determinations of the osmotic pressure of expressed sap give no indication of the diffusion-pressure deficit of the tissues from which the sap was taken. In this instance, however, it may reasonably be assumed that at full turgor the wall pressure just equaled the osmotic pressure and that therefore the diffusion-pressure deficit was approximately zero. At the first permanent wilting point the wall pressure of the cells in the basal leaves was approximately zero, and the diffusion-pressure deficit was therefore approximately equal to the osmotic pressure. The diffusion-pressure deficit of the remainder of the plant top must have been equal to that of the lower

leaves and the osmotic pressure slightly higher than that of the basal leaves, since the upper leaves were at least partially turgid. It seems reasonable to assume that the diffusion-pressure deficit of the roots at the first permanent wilting point was somewhat, but not greatly, less than the osmotic pressure observed in the samples of sap expressed from the tops. At the ultimate wilting point wall pressure was probably zero, and the diffusion-pressure deficit was equivalent to the osmotic pressure. The marked increase in the osmotic pressure of sap, as the soil dried out from field capacity to the ultimate wilting point, undoubtedly resulted principally from loss of water by the tissue rather than from an increase in solutes. The average moisture content of plant tops, expressed as grams of water per gram of dry matter, was in plants at full turgor, 10.49; at the first permanent wilting point, 8.88; and at the ultimate wilting point, 5.24. The freezing-point determinations, then, indicate that the force with which water in soil at the root surface was held at the first permanent wilting point amounts to somewhat less than 9.1 atmospheres, and at the ultimate wilting point to about 22.2 atmospheres. These values, expressed in terms of soil-moisture tension, would indicate that, in soils practically free of salts, the tension at the first permanent wilting point may be somewhat less than 9,400 cm. of water (pF 3.97), and at the ultimate wilting point the tension may be about 22,900 cm. of water (pF 4.36).

From a comparison of the general level and change of slope of the curves in figure 3, it appears that at soil-moisture percentages between the moisture equivalent and the first permanent wilting point, the osmotic pressure of sap of the plants held in nonhumidified air was affected by transpiration. The state of turgor of leaves and the osmotic pressure of sap of these plants at soil moisture 2 or 3 percent above the first permanent wilting point were about the same as that of the plants from the humid air with soil moisture at the first permanent wilting point. When the soil moisture of both series of plants was at the first permanent wilting point, the difference in turgor and in osmotic pressure of sap of the two groups was quite large. With the decrease in soil moisture from field capacity to the first permanent wilting point, the increase in the osmotic pressure of the sap was about 5 atmospheres in the plants held in nonhumidified air but only 2.5 atmospheres in plants held in humid air. Apparently, when plants are held under conditions that permit water loss, even though the rate of loss is relatively low, as it was in this instance, there is some lag of absorption behind transpiration. At soil-moisture percentages near or in the wilting range the departure from equilibrium between the forces acting on the water in plant and soil may be large. Apparently, to obtain reliable values for the first permanent wilting point, it is essential that the cultures be handled in such a manner that the lower leaves just remain flaccid and that the second pair recover at least partial turgor when the forces acting on water in plant and soil are at approximate equilibrium.

When such conditions of equilibrium exist, the force with which water is held by soil at the root surface is, at the first permanent wilting point, probably nearly equal to the diffusion-pressure deficit of the basal leaves, and at this stage the turgor of cells in the growing regions has been reduced to the point at which further elongation does not take place or proceeds very slowly. First permanent, or irre-

versible, wilting is then a fairly well-defined stage in the progressive changes that occur in young healthy sunflower plants grown under certain specified conditions, and this stage is reached by all such plants in the same soil at approximately the same soil-moisture percentage. It should be clear, however, that the well-defined end point at the first permanent wilting point of the soil is well defined only so far as the response of the plant is concerned and only if the plant-soil system is at approximate equilibrium. The first permanent wilting point does not mark a point of abrupt change in the state or properties of the water in the soil. It has been supposed by some writers that there is a sharp break in the continuity of the water columns or films in the soil at the first permanent wilting point. Maximov (*11, p. 79*) suggested that ". . . even with the slowest rate of water absorption, the moment at last arrives when the water films in the drying soil are ruptured, and the water loses its mobility; this moment corresponds to the wilting coefficient." The fact that an appreciable amount of water is extracted by the plant at soil-moisture percentages between the first permanent wilting point and the ultimate wilting point indicates that there is not an abrupt cessation of water movement at the first permanent wilting point.

It is highly probable that root extension as well as stem elongation is negligible at soil-moisture percentages below the first permanent wilting point and that the extraction of moisture in the wilting range is dependent almost entirely on water movement to the roots. Diffusion of water vapor probably accounts for a part of this movement, but the results recently obtained by Richards (*13*) and Richards and Weaver (*14*) indicate that moisture transfer at rates higher than can be accounted for by gaseous diffusion occurs in soils at moisture percentages in the wilting range when pressures at the membrane surface are comparable in magnitude to the estimated diffusion-pressure deficits of roots in soil in the wilting range. Richards found that at a pressure of 16 atmospheres the moisture content of 5- to 10-mm. layers of soil was reduced from saturation to moisture percentages in the wilting range in 24 to 36 hours.

It has also been supposed that at the first permanent wilting point the force with which water is held by the drying soil increases so sharply that significant amounts of water cannot be extracted by the plant beyond this point, and that consequently the stage or degree of permanent wilting selected as the end point in wilting-point determinations is of slight importance. Vapor-pressure curves of soils are usually shown for a wide range of soil-moisture percentages, such as that from the moisture equivalent to the air-dry or oven-dry state, and over such a range the change in the slope of the curve appears sharp indeed in the region of the first permanent wilting point. It may be noted, however, from figure 3 that the break in the osmotic-pressure curve also is relatively sharp in the same region and that the increase of about 13 atmospheres in osmotic pressure, and presumably in diffusion-pressure deficit, between the first permanent wilting point and the ultimate wilting point results in the absorption of appreciable amounts of water at soil-moisture precentages below the first permanent wilting point, although it is true that below that point the rate of absorption is low.

WILTING RANGE AND ITS RELATION TO AVAILABLE RANGE, MOISTURE
EQUIVALENT, AND PERCENTAGE OF SOIL COLLOIDS

The first permanent and the ultimate wilting points of the soils
used in this study are shown plotted against the moisture equivalent
in figure 4.

In general, the magnitude of the wilting range increases with in-
creasing fineness of texture, as indicated by the moisture equivalent,
but this relation is by no means consistent. Some soils of nearly the
same moisture equivalent vary appreciably in magnitude of the wilting
range.

The differences in magnitude of the wilting range are apparently
largely determined by the moisture-retaining characteristics of the
different soils at soil-moisture tensions in the wilting range, though
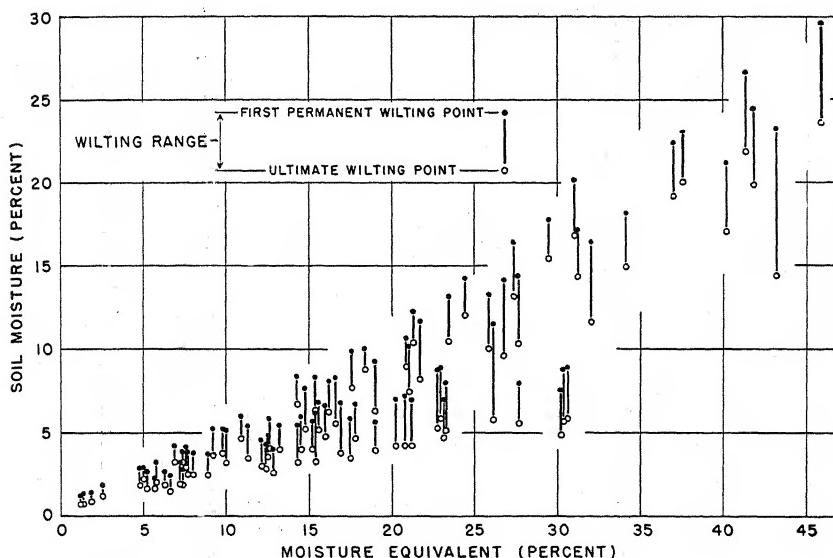
FIGURE 4.—First permanent wilting point and ultimate wilting point of different
soils varying in texture from sands to clays. The wilting range of each soil is
represented by the vertical distance between the first (dot) and ultimate wilting
(open circle) points.

other factors, such as osmotic pressure of the soil solution, are probably
involved. The moisture-retention characteristics have been de-
termined on duplicate samples of many of these soils by Richards
and Weaver (*14*). The slope of their moisture-retention curves at
tensions in the region of the wilting range shows that, in general, for
a unit change in tension the magnitude of the change in moisture
percentage of the soil increases with increasing fineness of texture,
but this relation, like that between wilting range and texture, is not
consistent. That is, the moisture-retention curves of some soils of
about the same moisture equivalent cross in the region of the wilting
range.

The soil moisture within the wilting range provides the plant with an emergency reservoir that enables many species of plants to survive periods of prolonged drought or to mature seed after vegetative growth has ceased as a result of water shortage. With the reduction in the rate of absorption, which occurs near the first permanent wilting point, and the severe water shortage that follows, the various mechanisms by which transpiration is greatly reduced, such as stomatal closure and abscission of leaves, are set in motion; but after these changes are in progress there still remains the available water of the wilting range, which may be slowly absorbed over a relatively long period. Since the magnitude of the wilting range of different soils, even though they may be of similar texture, varies widely, it seems likely that this characteristic of a soil may be of some importance among those factors that effect the survival of plants during periods of drought. It is true, however, that the differences in magnitude
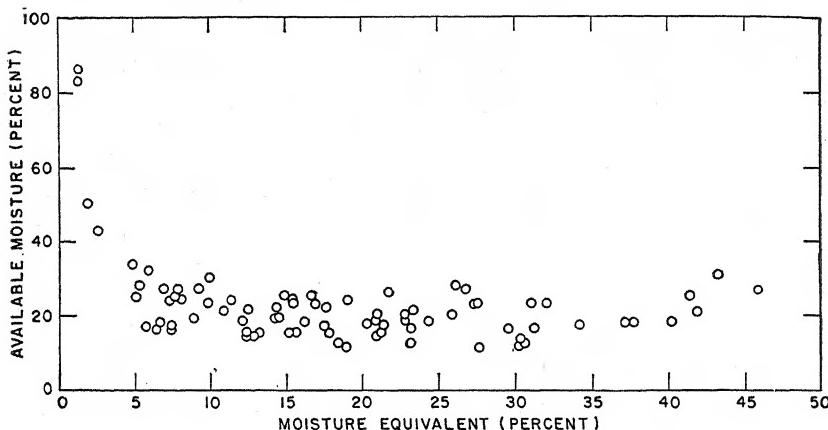
FIGURE 5.—Approximate percentage of available moisture held in wilting range of soils of different moisture equivalents.

of the wilting range of coarse- and fine-textured soils would be less, because of the greater volume weight of the coarse-textured soils, if the wilting range were expressed as moisture per unit volume of soil rather than as percentage of dry weight.

Perhaps the importance of the wilting range in the water economy of the plant may be evaluated most readily by a comparison of the wilting range and the range of soil moisture available to the plant. The approximate percentage of the available moisture that is held in the wilting range of soils of various moisture equivalents is shown in figure 5. The approximate available range is assumed to be the moisture held between the ultimate wilting point and the moisture equivalent. This assumption is not strictly correct, since the moisture equivalent is slightly higher in clays and appreciably lower in sands than the field capacity; but the moisture equivalent has been widely used as an approximation of field capacity, and, since it may be considered as a soil-moisture constant, determined by a standardized method, whereas the field capacity is not a constant but is affected by the peculiarities of the particular profile from which the sample

was taken, the moisture equivalent seemed to be a more suitable basis for a generalized comparison than the field capacity. It should be noted, however, that below a moisture equivalent of about 5 the discrepancy between the moisture equivalent and field capacity increases sharply; according to Browning (7), in soils of very coarse texture the field capacity may be two or more times the moisture equivalent. This, doubtless, accounts for the apparently high proportion of the available moisture held in the wilting range of the four soils of lowest moisture equivalent (fig. 5). Most soils of importance in agriculture fall within the textural range corresponding to moisture equivalents of 5 to 45, and in this range the proportion of the available moisture in the wilting range varies from about 11 to about 30 percent, averaging roughly 20 percent. It is clear that the proportion of the total available moisture that is held in the wilting range is great enough in most soils to be of significance in any consideration of the influence of soil-moisture shortage on the behavior of plants.

While it is not supposed that there is a sharply defined transition in the properties of the soil water or in the mechanism of water absorption by the plant at the first permanent wilting point or the ultimate wilting point, it seems possible to make a fairly well-defined division of soil moisture, so far as its availability to plants is concerned, into (1) that available for vegetative growth, (2) that available for maintenance of life under conditions of greatly reduced turgor, and (3) that unavailable to plants.

Because of the time and labor involved in making direct determinations of the wilting point of soils, several investigators have proposed less laborious, indirect methods, based upon physical measurements and not involving the growing of plants in the soil. The most widely used of these methods, that of Briggs and Shantz (6), is based upon an expected constancy of the ratio of the moisture equivalent to the first permanent wilting point, which they found to be approximately 1.84 in about 28 soil types investigated. Veihmeyer and Hendrickson (18) and, later, others called attention to the fact that in many soils the ratio of moisture equivalent to wilting point deviates widely from the value 1.84, observed by Briggs and Shantz.

Since the ratios reported by some workers (18) were nearly all above 1.84 and those reported by others (15) were nearly all below 1.84, it seemed possible that some of the variations in ratio observed may have resulted from variation in the degree of wilting judged by the different workers to be permanent wilting. In the present investigation, since the entire wilting range was determined, the possible importance of this factor may be evaluated from the data shown in figure 4. It is apparent from these data that wide variation in degree of permanent wilting could cause appreciable variation in the ratio of moisture equivalent to wilting point; but even the widest variation in this respect could account for only a part of the observed variability in ratio of these soils, since the ultimate wilting point of some lies far above the first permanent wilting point of others of about the same moisture equivalent. These data fully confirm the conclusion of Veihmeyer and Hendrickson (18) and others, that the ratio of the moisture equivalent to the wilting point of different soils varies widely. At first permanent wilt the moisture content of soils within the narrow

moisture-equivalent range of 30 to 31 varied from 7.6 to 20.2 percent and the ratio of the moisture equivalent to the first permanent wilting point varied from 3.97 to 1.53. The highest ratio of the moisture equivalent to the first permanent wilting point obtained in any of the soils (3.97) was obtained with a sample classified as Coachella fine sand, though it had moisture equivalent of 30.2 and mechanical analysis showed 30 percent silt and 40 percent colloid; whereas the lowest ratio, 1.08, was obtained with a coarse sand that had a moisture equivalent of 1.3 and was probably very low in percentage of colloid.

In an investigation of the relation of soil-moisture properties to texture in a large number of soils of the Okanagan Valley, British Columbia, Wilcox and Spilsbury (*20*) found that the values obtained for the wilting coefficient by the cohesion method of Bouyoucos (*5*) were closely correlated with the percentages of colloid as determined
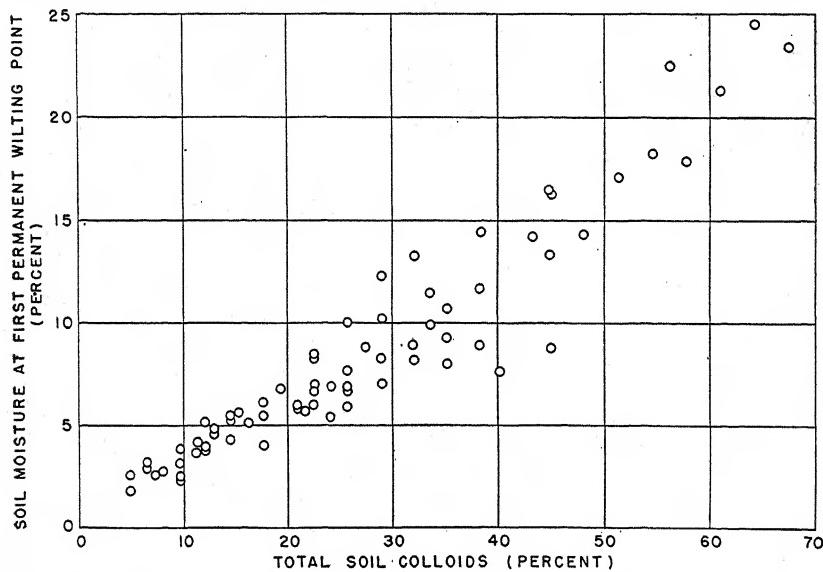
FIGURE 6.—Relation between percentage of soil colloids and first permanent wilting point of different soils.

by the hydrometer method of Bouyoucos (*4*). They concluded that the wilting coefficient may be calculated from the equation

$$\text{Wilting coefficient} = 0.12 \text{ percent colloid} + 1.338$$

with only a relatively small amount of error, though they warn that this equation may not prove satisfactory for use with soils formed under climatic conditions differing widely from those of the Okanagan Valley.

The relation of the first permanent wilting point to the percentage of colloids, as determined by the hydrometer method,[4] of most of the soils used in the present investigation, is shown in figure 6. It is obvious that the relation is not constant. For the 71 soils the average

ratio of percentage of colloids to first permanent wilting point was 3.21, but the ratios ranged from 1.85 to 5.29.

Although, in a general sense, with increasing fineness of texture there is an increase in the moisture equivalent, the percentage of colloids, and the wilting range, it is apparent that there are differences in the physical make-up of these soils that are not reflected in the moisture equivalent or the percentage of colloids.

A striking illustration of the failure of the moisture equivalent or percentage of colloids to reflect accurately the moisture-holding properties of the soil is that of several samples of soil from recent alluvial formations in the Coachella Valley, Calif. These soils are composed of 26 to 29 percent sand, 29 to 38 percent silt, and 32 to 45 percent colloids. The values for the first permanent wilting point range from 7.6 to 8.9 percent and for the moisture equivalent from 30.2 to 30.6 percent. It appears that, in relation to either the moisture equivalent or the percentage of colloids, these soils hold exceptionally low amounts of water at the first permanent wilting point.

It is possible that the movement of water through the soil to root surfaces, the number or distribution of roots, or perhaps the operation of other factors that might affect the value of the wilting points is related to variations in the texture and structure of the soil and in the composition of the soil solution; but it is highly probable that a large part of the variation in the ratios of the moisture equivalent or the percentage of colloids to the first permanent wilting point can be attributed to variations in the relative amounts of water retained by different soils with variations in soil-moisture tension. For example, the statement previously made regarding the moisture-holding properties of the Coachella Valley soils, as compared with others of similar moisture equivalent or percentage of colloids, may be generalized as follows: Soils A and B retain about the same amount of water at low moisture tension (moisture equivalent), while at high tension (wilting range) soil A retains less water than soil B.

Olmstead (*12*) found pronounced variations in the relative amounts of water retained by soils that had been saturated and then centrifuged in fields of 1,000 and 300,000 gravity, and more recently Richards and Weaver (*14*), using their porous-plate and pressure-membrane apparatus, found that the relative amounts of moisture retained by soils may vary over the entire range of moisture tensions from that at field capacity to that at the ultimate wilting point. Their curves showing the moisture retained by different soils at tensions ranging from about 2 to about 20,000 cm. of water are, however, more nearly parallel in the tension range above 3,000 cm. of water than in the range below this value. In their investigation, Richards and Weaver used duplicate samples of most of the soils employed in the present study. Their comparisons of the moisture percentage of these soils at a moisture tension equivalent to 15 atmospheres with the moisture percentages at the two wilting points suggest that the relation of the first permanent wilting point or of the ultimate wilting point to the 15-atmosphere percentage is more nearly constant than the relation of either of the wilting points to the moisture equivalent or to the percentage of colloids. The 15-atmosphere percentage would, therefore, probably serve as a more reliable basis for the indirect determination of the wilting range than would the moisture equivalent or the percentage of colloids.

DISCUSSION AND CONCLUSIONS

The idea that a soil has a relatively definite wilting coefficient, or first permanent wilting point, has been seriously questioned by various writers from time to time since Briggs and Shantz (6) first published the results of their work on this subject. Questions as to the reality of a first permanent wilting point have been raised, possibly as a result of a misunderstanding of (1) the meaning of the term "first permanent wilting point," (2) the precautions essential for the reliable determination of its value, and (3) the use to which it may be put in interpreting the behavior of plants in relation to soil-moisture conditions in the field.

The first permanent wilting point might be defined as that percentage of soil moisture at which the forces acting on the water in the soil are at approximate equilibrium with the forces acting on the water in a plant which is at that stage of turgor at which vegetative growth practically ceases. At moisture percentages above the first permanent wilting point, temporary wilting, or even death of much of the leaf surface, may result from prolonged exposure to conditions that produce intense transpiration. As long, however, as the moisture content of the soil is above the first permanent wilting point, vegetative growth of the plant may be resumed if transpiration is reduced to a negligible rate and other conditions are kept favorable for growth.

The results of the investigations of Caldwell (8) and of Shive and Livingston (16) have frequently been cited as proof that the moisture content of the soil at which permanent wilting takes place is affected by the environmental conditions existing during the wilting process. In the course of the present work, however, it was demonstrated many times that wilting plants rapidly or slowly made no significant difference in the soil-moisture percentage at first permanent wilt, provided the plants were placed in a humid chamber before the basal leaves were injured and before the second pair of leaves was permanently wilted. In the present investigation the plants were protected from rapid water loss after the first one or two temporary wiltings, because it was found that under conditions of low transpiration fewer transfers back and forth from the greenhouse bench to the humid chamber were required than if the plants were subjected to intense water loss. It seems possible that in reported instances of appreciable influence of the environment during wilting on the moisture content of the soil at permanent wilting the leaves may have been injured before the plants were placed in the humid chamber, that the degree of wilting may have been allowed to go beyond that of first permanent wilting before the plants were placed in the humid chamber, or that the roots may have been injured by high soil temperatures. Caldwell (8) reported root injury, which he attributed to desiccation, in some of his cultures. In the present work root injury, apparently resulting from high soil temperatures caused by exposure of several culture cans to direct sunlight on a hot day, was observed when the cultures were at soil-moisture percentages above the first permanent wilting point. Root injury that could be attributed to desiccation alone, however, was never observed in cultures dismantled

at the first permanent wilting point; and even at the ultimate wilting point most of the root system appeared to be alive.

The reality of a first permanent wilting point (wilting coefficient) of the soil has been questioned by some writers because soil-moisture conditions and wilting often appear not to be very closely related in the field. Soil within the root zone of plants in the field may be found to vary in moisture content from percentages well above the first permanent wilting point to percentages well below it; at the same time, depending upon the species and the weather conditions, some plants may show severe wilting and others no visible signs of water shortage. Wilting of a normal uninjured plant when the soil in the entire root zone is above the first permanent wilting point is temporary, and the plant may recover turgor and continue vegetative growth when transpiration becomes sufficiently reduced. On the other hand, it is not unusual to find under field conditions that the moisture content of the soil in a part of the root zone is below the first permanent wilting point before permanent wilting occurs and vegetative growth ceases. When temporary wilting occurs or a comparable state of turgor is reached in plants that do not show wilting, the diffusion-pressure deficit of the roots may soon become great enough to make possible absorption of water from soil at moisture percentages below the first permanent wilting point. This is likely to occur only in the zones of highest root concentration, while the soil in zones of lower root concentration is still above the first permanent wilting point. Then, when transpiration is greatly reduced, as at night, the plant may recover sufficient turgor, as a result of water absorbed by roots in soil at moisture percentages above the first permanent wilting point, to resume vegetative growth.

A prerequisite to the rational application of the conception of a wilting range of the soil to field conditions is an understanding of the manner and pattern of water extraction by the plant from the soil. From extensive field work relating to irrigation problems, there has been formulated the following picture of the typical pattern of root distribution and the sequence of events in the extraction of water from soil initially wet to soil at the ultimate wilting point. While the distribution of roots varies greatly with species and soil, the concentration of absorbing roots is typically greatest in the upper part of the root zone and near the base of the plant and decreases with soil depth or distance from the plant. Extraction of water is most rapid in zones of highest root concentration and most favorable conditions of temperature, aeration, and other environmental factors. When the moisture content in the zone of highest root concentration has been reduced to the first permanent wilting point, extraction in this zone does not cease, but the rate falls off sharply and the total water absorption rate of the plant decreases. As the total absorption rate and the turgor of the plant decrease, the diffusion-pressure deficit of the root system as a whole increases, the soil-moisture percentage is lowered into the wilting range progressively in zones of lower and lower root concentration, and, finally, as the severity of wilting increases, the soil-moisture percentage is reduced to the ultimate wilting point progressively from zones of highest root concentration to zones

of lower root concentration. By the time the plant in the field dies as a result of desiccation, the soil-moisture percentage in a large part of the root zone may have been reduced to the ultimate wilting point. Soil at the extremities of the root system, however, may still be well above the first permanent wilting point. The plant dies, not because water absorption has absolutely ceased, but because the rate of absorption finally lags too far behind the rate of loss to support life.

The significance of the first permanent wilting point or of the ultimate wilting point in the interpretation of field data lies in the fact that these points serve as reference values to which soil-moisture percentages may be related in making estimates of the amount of water in the root zone of a plant that is available for vegetative growth or for the maintenance of life.

It is hardly to be hoped that plant responses to soil-moisture conditions in the field may be very sharply defined. By the time the plant shows any visible effect of moisture shortage the moisture content of the soil in different parts of the root zone may vary widely; and atmospheric conditions, depth and distribution of roots, nutrition of the plant, the differences in susceptibility of different species to injury by temporary desiccation, and doubtless other factors affect the response of the plant in the field to soil-moisture conditions. By actual experiment with a given species of plant and set of soil conditions, however, it is possible to predict with a fair degree of accuracy the response that a plant will make when the moisture content of the soil in various proportions of the root zone is reduced to percentages in the wilting range under similar conditions of soil and climate.

SUMMARY

The range of soil-moisture percentages through which plants undergo permanent wilting has been termed the "wilting range of the soil." If the sunflower is used as the test plant, the upper end of this range, the first permanent wilting point, is marked by permanent wilting of the basal leaves, and the lower end of the range, the ultimate wilting point, is marked by complete permanent wilting of the apical leaves.

A standardized procedure for making wilting-range determinations is described, and the results obtained on about 80 soils are presented. At soil-moisture percentages near or in the wilting range even a low rate of water loss from the plant had an appreciable effect upon the osmotic pressure of the sap and upon the turgor of the plant. A decrease in soil moisture from field capacity to the first permanent wilting point caused, in plants in dry air, an increase of 5 atmospheres in the osmotic pressure of the sap and, in plants in humid air, an increase of only 2.5 atmospheres. The changes in osmotic pressure of plants in humid air indicate that the diffusion-pressure deficit of the plant was somewhat less than 9 atmospheres at the first permanent wilting point and about 22 atmospheres at the ultimate wilting point.

The proportion of the available moisture in the wilting range is great enough to be of considerable significance in investigations of the effect of soil-moisture shortage on plants. Of the moisture held between the ultimate wilting point and the moisture equivalent, the

proportion held within the wilting range of the soils investigated varied from about 11 percent to about 30 percent and averaged about 20 percent.

It was found, in agreement with other work, that the ratio of the moisture equivalent to the first permanent wilting point or to the ultimate wilting point is not constant. It was also found that the percentage of soil colloids, which has recently been used as a basis for calculation of the wilting point, would not serve as a reliable basis for calculation of the wilting points of the soils used in this study.

As related to plant behavior, soil moisture may be classified as (1) moisture available for vegetative growth, (2) moisture in the wilting range, and (3) moisture unavailable to plants.

In field work the first permanent wilting point and the ultimate wilting point may be used as reference values for estimating, respectively, (1) the amount of moisture in a soil that is available for vegetative growth and (2) the amount available for mere maintenance of life.

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TEMPERATURE IN RELATION TO DEVELOPMENT AND CONTROL OF BLUE MOLD (PERONOSPORA TABACINA) OF TOBACCO [1]

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INTRODUCTION

Blue mold, caused by *Peronospora tabacina* Adam, is known as a cool-weather disease of tobacco (*Nicotiana tabacum* L.). In Georgia it has been moderately active during periods with temperature between 30° and 35° F. Epidemic outbreaks, however, have usually occurred with minimum temperatures between 55° and 62°, and minimum temperatures between 65° and 70° have caused the disease to disappear. The inhibitory effects of only moderately high temperatures led the writers to study the feasibility of blue mold control through temperature regulation.

VIABILITY OF SPORES

To understand better the effect of temperature, it has been necessary to consider separately such important phases of disease activity as spore production, spore germination, and leaf infection. It is during the period of spore production that the most conspicuous stage of disease development occurs. After germination and infection have taken place, the mycelium grows through the leaf tissues for about 6 days without causing noticeable injury. The conidiophores then grow out through the stomata of the lower leaf surface about dawn, and within a few hours the affected leaf tissues begin to wilt and die. Obviously it was important to fix the period during which spores are mature and viable. This was done by selecting and marking, during the day, leaf lesions that had not yet sporulated but would probably sporulate the following morning. These lesions were then examined at intervals, and, after sporulation was first observed, germination tests were made from time to time. The results of such a test are given in table 1.

The data in table 1 show that only a few lesions had produced mature spores by 4 a. m. The majority of lesions had mature spores by 5 a. m., indicating the rapidity with which conidiophore growth and spore formation proceed. However, the fact that 7 of the 20 germination tests with spores collected at 5 a. m. gave values of 0 or 1 percent indicates that many of the spores were still immature. The highest mean germination was obtained with spores collected at 6 a. m. By 11 a. m. most of the spores were dead but 7 collections gave germination counts between 20 and 50 percent; and even as late as 5 p. m. a few of the spores were still viable.

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TABLE 1.—*Maturity and viability of spores of Peronospora tabacina collected at different hours of the day*

Time collected	Collections with spores apparently in the condition indicated			Spores germinating in different collections	Spores germinating (mean)
	Mature	Immature	Not formed		
	Number	*Number*	*Number*	*Percent*	*Percent*
4 a. m	1	4	15	0; 0; 9; 11; 24	9
5 a. m	15	5	0	0; 0; 0; 0; 1; 1; 1; 10; 13; 15; 17; 36; 45; 47; 56; 56; 80; 81; 86; 93.	32
6 a. m	16	4	0	3; 4; 4; 6; 7; 12; 21; 25; 35; 39; 47; 57; 80; 82; 85; 87; 88; 88; 91; 92.	48
8 a. m	20	0	0	0; 0; 1; 4; 4; 4; 8; 11; 17; 21; 22; 29; 32; 33; 42; 68; 73; 74; 77; 83.	30
11 a. m	20	0	0	0; 0; 0; 0.5; 1; 1; 1; 2; 2; 3; 3; 4; 12; 21; 21; 27; 31; 37; 40; 50.	13
5 p. m	20	0	0	0; 0; 0; 0; 0; 1; 1; 1; 1; 2; 2; 3; 4; 4; 4; 5; 5; 6; 7.	2

EFFECT OF TEMPERATURE ON SPORULATION

To obtain definite data on the relation of temperature to sporulation in the spring, leaves that appeared ready to sporulate were picked in the plant beds and held for 24 hours in a saturated atmosphere at different temperatures. In this preliminary experiment the leaves held at 40°, 50°, 69°, and 76° F. did not sporulate. A majority of those held at 64° and a lesser number of those held at 61° did sporulate. This result was surprising, because many temperature records made in plant beds have correlated heavy sporulation with a night temperature of about 60°. However, under natural conditions a night temperature minimum of 60° would be associated with a day temperature maximum of 75° to 80°. Tests were made, therefore, in which sporulation data at constant and alternating temperatures were compared.

The data in table 2 indicate that a temperature of 77° F. followed by 60° was more favorable for sporulation than 60° maintained continuously. No sporulation occurred when the temperature did not fall below 70°.

TABLE 2.—*Effect of constant and alternating temperatures on sporulation of Peronospora tabacina*

Temperature (°F.)	Exposure	Leaves sporulating	Average sporulation area
Constant temperature:	*Hours*	*Percent*	*Square inches*
50	24	12	0.16
60	24	35	.32
64	24	68	1.55
Alternating temperatures:			
77 / 50	7 / 17	32	.68
77 / 60	7 / 17	79	3.38
77 / 64	7 / 17	54	2.16

When plants were grown in the greenhouse in winter under various temperature conditions and with high humidity, very different results were obtained. During January and February heavy sporulation occurred at 82.5° F. in one experiment and at 75° in two others.

Furthermore, the disease was repeatedly observed to develop freely in the greenhouse during midwinter with minimum temperatures of 70° to 80°, indicating that the temperature responses of the blue mold were not the same in the plant beds in the spring as in the greenhouse during the winter.

Study of this situation has led to the conclusion that these differences are related to light intensity. Bed-grown plants under the strong sunlight of spring had comparatively tough leaves, whereas plants grown in the greenhouse under the weak sunlight of winter had leaves that were tender and succulent. These winter plants were very much more disease-susceptible, and sporulation and disease development took place freely at minimum temperatures of 70° to 80° F. In the more resistant spring plants, sporulation and disease development did not occur at minimum temperatures in the 70° to 80° range. The fact that shading increases the severity of blue mold attack requires no proof, as it is a common observation that the shaded parts of plant beds are the most severely diseased. Furthermore, in parts that were shaded artificially the disease had remained active in the late spring for as much as 2 weeks after all development had ceased in adjacent unshaded parts of the bed. The effect of shading was also studied by growing plants under different thicknesses of cloth for different periods of time. Prolonged shading (3 to 4 weeks), which affected the type of growth, producing tender, succulent leaves, greatly increased sporulation and disease damage. However, growing plants in full sunlight, inoculating them, and then shading them for a week resulted in only a slight increase in disease development. The shading experiments were so arranged that differences in humidity were slight.

EFFECT OF TEMPERATURE ON SPORE GERMINATION

Germination tests with blue mold spores have given extremely variable results, as shown in table 1. This has raised the question as to what effect temperature might have on percentage of germination. Data are presented in table 3 on spore collections made at different hours on two different days.

TABLE 3.—*Effect of temperature on spore germination of Peronospora tabacina*

Spore series and time of collection	Germination at indicated temperature (° F.)												Optimum germination temperature
	35	40	50	60	63	64	69	71	73	77	79	82	
	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	° F.
Series 1:													
5 a. m.	0	2	33	7	------	23	------	49	72	------	30	8	73
6:30 a. m.	20	15	7	4	------	13	------	55	84	------	21	19	73
8 a. m.	16	21	19	10	------	90	------	60	30	------	80	17	64
9:30 a. m.	21	14	11	19	------	30	------	39	11	------	41	2	79
11 a. m.	5	18	26	10	------	33	------	------	16	------	9	3	64
Series 2:													
6 a. m.	95	95	95	9	10	------	2	------	60	2	------	------	35–50
7:30 a. m.	98	98	95	50	23	------	27	------	19	25	------	------	35–40
9 a. m.	37	46	84	12	13	------	9	------	21	16	------	------	50
11 a. m.	50	97	98	44	27	------	13	------	73	84	------	------	50
2:30 p. m.	48	33	95	40	5	------	8	------	2	8	------	------	50

The data in table 3 offer some interesting contrasts. It is evident that the same spore collection gave widely different germination percentages at different temperatures. Thus, in the series 1, 6:30 a. m. collection there was 7 percent germination at 50° F. and 84 percent at 73°. On the other hand, in the series 2, 6 a. m. collection there was 95 percent germination at 50° and 60 percent at 73°. There was no consistent optimum temperature for spore germination, and the optimum for each separate collection is listed in the last column of table 3. In series 1 optimum values ranged from 64° to 79° and in series 2 from 35° to 50°. Thus, there was variability in the responses of collections made on the same day and a complete shift in the temperature responses of collections made on two different days. Further germination tests were made on five other series of spore collections, each on a separate day, conducted in the same manner as those reported in table 3. In three series, germination was favored by cool temperatures (35°–50°), and in two by warm temperatures (64°–79°). In no experiment was any germination of spores obtained with temperatures of 85° or above.

Many factors besides temperature during germination affect the viability of spores. The spores produced on young seedlings (3 to 4 weeks old) have generally germinated much more poorly than spores produced on the leaves of plants 6 to 8 weeks old. Moreover, the temperature conditions that prevail while spores are being formed may have a marked effect on spore viability. Germination tests were made on spores produced under the different temperature conditions indicated in table 2, and the results were as follows: Maximum germination (94 percent) was obtained with spores produced at a temperature of 77° F. by day and 60° by night, whereas minimum germination (less than 1 percent) was obtained with spores produced at 50° maintained continuously. In general, abundant spore production has been associated with high viability.

Temperature is also a factor in determining the longevity of spores. Experiments were conducted in which the spores were stored in a saturated atmosphere at different temperatures. As would be expected the results showed that spores held at the cooler temperatures (35° and 40° F.) survived the longest. Actual freezing did not prove harmful; freshly sporulating leaves were held for 9 days at 16°, and the spores removed at the end of that time germinated about 70 percent as well as the spores of the same collection did originally. Spores stored at 32° did not keep nearly so well as those stored at 16°. The ability of the spores to live for days in a frozen condition was reported by Angell and Hill (*2*),[2] and this ability may have been an important factor in the recent spread of blue mold from the western to the eastern part of the United States.

EFFECT OF TEMPERATURE ON PLANT INFECTION

To obtain infection data, tobacco plants were sprayed with a suspension of spores in the early morning and then held in a saturated atmosphere at different temperatures for 24 to 72 hours. After this, they were removed to an open greenhouse where conditions favored disease development. Notes were taken at the end of 9 days, i. e.,

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[2] Italic numbers in parentheses refer to Literature Cited, p. 182.

before secondary infection had become a factor.   No experiment was made to determine whether temperatures at the time of inoculation affected the latent period, or length of time required for symptoms to appear.   Figure 1 shows typical results.   Incubation at 35°, 40°, 50°,
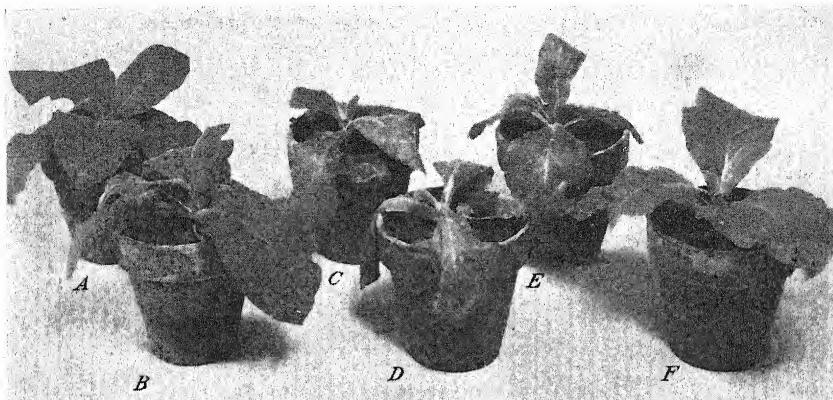
FIGURE 1.—Temperature in relation to blue mold infection.   Plants were inoculated and then incubated for 48 hours at the following temperatures: *A*, 40° F.; *B*, 50°; *C*, 64°; *D*, 75°; *E*, 80°; *F*, 90°.   Temperatures of 64° and 75° favored disease development.

and even 60° F. resulted in slight disease development; at 64°, 70°, and 75°, infection was severe.   No disease development was obtained after incubation at 85° or more, though abortive lesions were observed. In several of these experiments, it was possible in the early stages of disease development to count separate lesions.   Such counts showed the following number of lesions per plant: With incubation at 35° and 40°, no lesions; at 50° and 62°, 14 to 16 lesions; at 64° and 70°, 25 to 35 lesions; at 74° and 77°, 85 to 89 lesions.   Infection thus was favored by temperatures distinctly higher than the temperatures favoring spore production.

CONTROL BY TEMPERATURE REGULATION

During the 5-year period from 1933 to 1937, inclusive, experiments were conducted each year, principally at Tifton, Ga., and at the Arlington Experiment Farm, Arlington, Va., with plant-bed units so arranged as to provide various minimum night temperatures.   Electric heating cables with thermostats were used to obtain uniform gradations of night temperatures.   During the day temperatures were not regulated.

Table 4 shows a representative set of data.

TABLE 4.—*Control of blue mold in the plant bed by temperature regulation at night*

Minimum night temperature (°F.)	Sporulating leaves on 250 plants	General condition of plants
	Number	
60–64	650	Defoliation complete.
65–69	300	Defoliation moderate.
70–74	1–	Trace of infection.
75–79	1–	Do.
Check (55–63)	566	Defoliation complete.

The temperatures in the check areas dropped to 55° F. on some nights, and the disease was actually less active than in the 60° to 64° plots. Disease development decreased with 65° to 69° and was almost completely inhibited at 70° to 74° and at 75° to 79° (fig. 2). Humidity
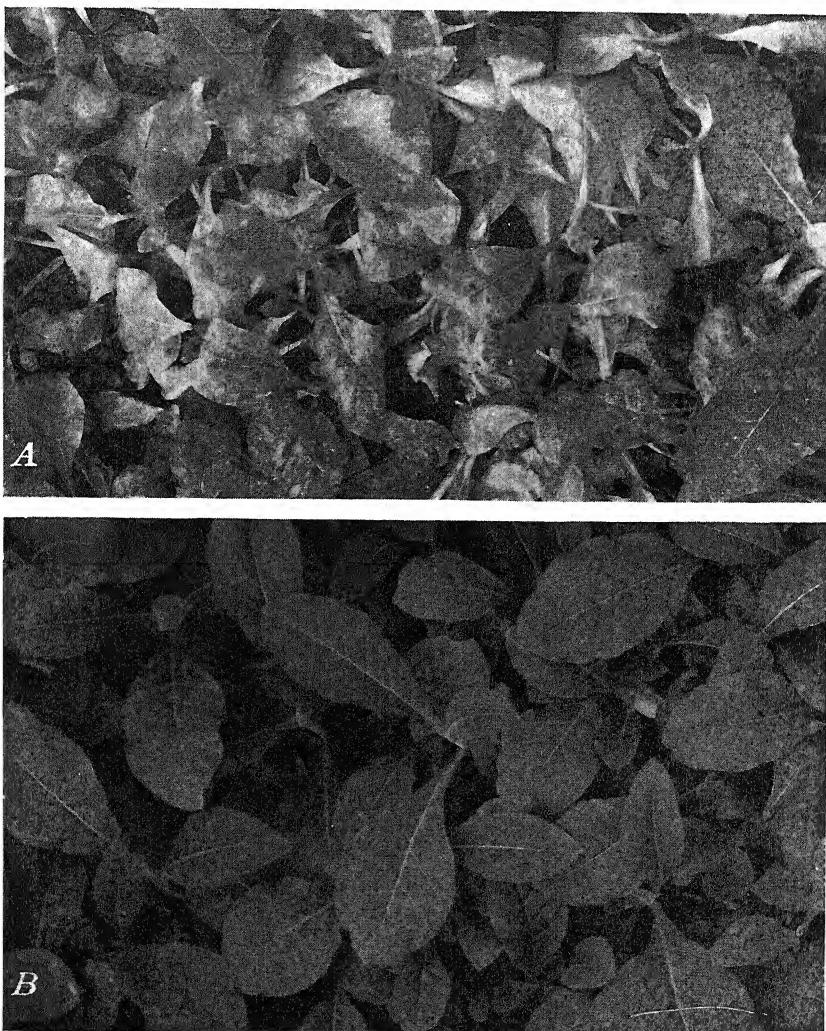
FIGURE 2.—Blue mold control by temperature regulation: *A*, Nonheated check; *B*, a section heated at night to maintain the temperature above 70° F. Severe blue mold developed in section *A*, while the plants in section *B* remained healthy.

reached saturation each night except with the very highest temperature.

In the work at Tifton, Ga., particular attention was paid to the development of practical plant-bed equipment for heat treatments. It was found that, because of the costs of installation and current,

electricity would be too expensive for the average tobacco grower. Figure 3 shows a flue-heated bed in which excellent blue mold control was obtained. The heat was provided by wood or coal fires, and the bed was covered with heavy muslin. Of the various heating methods tested, however, small oil heaters were found to be the most practical.

The fact that blue mold was regularly and effectively controlled in spring plant beds by maintaining night temperatures above 70° F., whereas the same treatment was completely ineffective in the greenhouse in midwinter, raised the question as to what would be the reaction of plants grown in beds sown in the autumn. In spring-sown plant beds, disease activity is checked abruptly by the first hot days of summer. However, when seed is sown about August 20 in the vicinity of Washington, D. C., blue mold becomes active about September 20
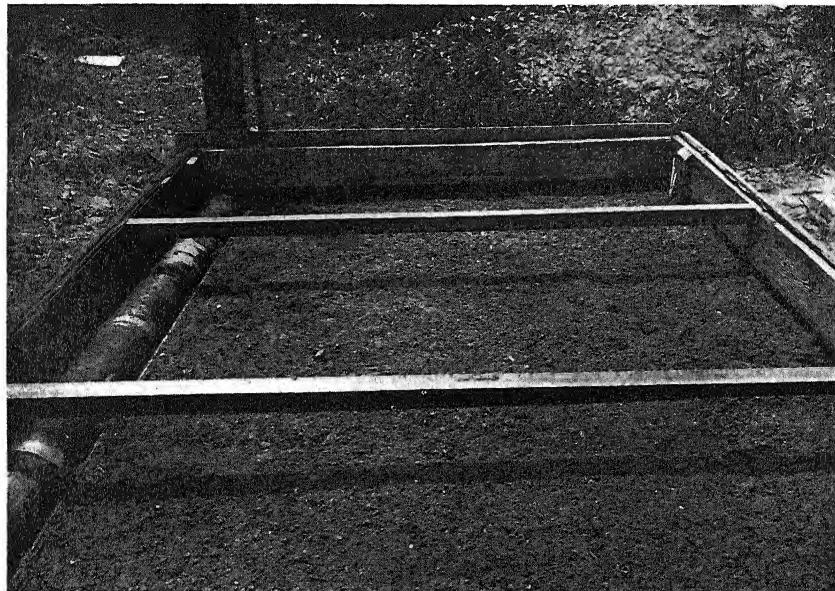
FIGURE 3.—A flue-heated tobacco plant bed. Excellent blue mold control was obtained in this bed in 1933.

After October 10 disease activity diminishes as temperatures drop. In the spring as plants grow the temperatures rise and the sun gets brighter, whereas in the autumn this sequence is reversed.

The first year that beds were sown in the autumn and temperature controls were established, September was warm and sunny. The plants resembled those grown in spring, and they reacted in the same manner to blue mold; that is, when the minimum night temperature was maintained above 70° F., effective blue mold control resulted. The autumn of the following year (1934) was very different. September was the wettest on record; and, owing to this excessive moisture and the cloudy weather, plant growth was succulent and soft. In this experiment there was abundant disease development at a night minimum of 70° to 75°, and the disease was almost equally active throughout the entire range of 60° to 75°. However, when the temperature

was raised to 85° to 90°, all disease development was promptly checked. To summarize briefly: In the spring sporulation is inhibited by night temperatures above 70°; in the autumn sporulation may or may not be inhibited by temperatures above 70°; and during the winter sporulation proceeds freely with night temperatures as high as 80°.

In studying the value of short treatments at temperatures above 85°, it seemed evident that such treatments would be most practicable during the day, when the heat of the sun would be an aid. The treatment periods, consequently, were limited to the period from 9 a. m. to 4 p. m. Except during the actual treatment period, the test plants were constantly exposed to reinfection and were held at temperatures favorable for blue mold development. The results from a number of tests are summarized in table 5.

TABLE 5.—*Control of blue mold by short treatments at high temperature*

Temperature (°F.)	Exposure per week	Blue mold development	Temperature (° F.)	Exposure per week	Blue mold development
	Hours			*Hours*	
90	12	Severe.	110	2	Moderate.
90	16	Do.	110	4	Slight.
90	48	Moderate.	110	8	Trace.
100	4	Do.	110	12	None.
100	8	Do.	Check		Severe to very severe.
100	12	Slight.			

In table 5 disease development designated as slight or less indicates practically perfect disease control, as figure 4 shows. Four hours per week at 110° F. effectively controlled blue mold, while a reduction of the temperature to 100° increased the time required for treatment to 12 hours. These results were obtained under severe disease conditions. In several experiments, 2 hours' treatment per week at 110° controlled
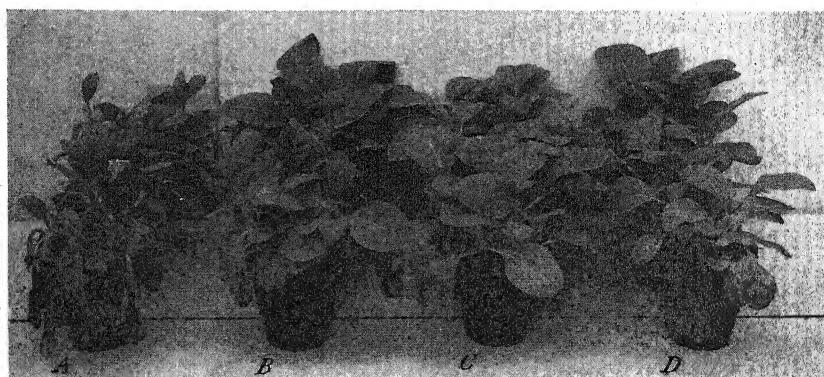
FIGURE 4.—Control of blue mold by short treatments at high temperatures: *A*, Untreated check, severe blue mold; *B*, 2 hours per week at 110° F., slight blue mold; *C*, 4 hours per week at 110°, no blue mold; *D*, 8 hours per week at 110°, no blue mold. Except during the brief treatment periods, plants were held under conditions favoring blue mold development and were constantly exposed to reinfection.

blue mold, but under the most severe conditions 4 hours were required. High-temperature heat treatment has been successfully used in large greenhouse units. The procedure there was to select a bright day and to leave the heat on and the ventilators closed. In the early spring it was possible to hold temperatures between 105° and 110° from 10 a. m. to 3 p. m., and such a treatment once a week has given very effective blue mold control.

High-temperature treatment was also used with success in cold frames with a large air capacity, but it did not prove safe in the usual sash bed because the very limited air space caused rapid fluctuation in temperature. Unless plants were extremely succulent, the only injury caused by 110° F. was a slight retarding of growth.

The high-temperature method did not prove practicable in regular commercial beds during the normal plant-bed season in Georgia, and effective control was obtained only in small tight beds covered with glass or glass substitutes. Heavy canvas covers were not suitable. It was not feasible to maintain the required uniformly high temperature on cold, windy, or cloudy days. Since very severe blue mold infection has occurred in Georgia during the coldest part of the winter, this treatment could be depended upon only during bright spring days or late in the plant-bed season. At such times the disease has not been very destructive and control was less essential than earlier in the season.

DISCUSSION

Pathologists generally have found rather definite and narrowly limited optimum temperatures for both the germination of fungus spores and the development of disease. The present studies show, however, that the optimum temperature for germination of *Peronospora tabacina* spores varied greatly with different collections. With some collections of spores the most favorable germination temperatures were 35° to 50° F., and with others 64° to 79°. No germination of spores was obtained at 85° or above, nor at 32° or below; so it is quite apparent that the optimum range was only a little less wide than the total range over which germination occurs.

The situation with respect to blue mold control by temperature regulation is also unusual. The writers reported (*4*) inhibition of sporulation and excellent control of blue mold by maintaining night temperatures above 70° F. These experiments were carried out in the usual spring plant beds and confirmed observations made by other workers (*1, 5*) that little if any sporulation occurs at temperatures above 68°. In fact, it is usual in the spring for the disease to disappear after a few nights with minimum temperatures between 65° and 70°. However, Armstrong and Sumner (*3*) reported sporulation with temperatures that remained above 80°, and in the experiments reported here the writers have found that blue mold can develop freely at a minimum temperature of 70° to 80° and produce spores abundantly as well. The essential condition for this high-temperature disease activity has been vigorous growth of the tobacco plants coupled with low light intensity, which results in very tender and succulent foliage. Thus, depending on the condition of the host plant, sporulation and disease development may or may not be inhibited by temperatures between 70° and 80°. Over a 5-year period (1933–37) the writers did not fail to obtain excellent blue mold control in repeated

tests by maintaining night temperatures above 70°. These experiments were all conducted in the usual spring plant beds. Experiments were also conducted in autumn-sown plant beds, and during 1 year, with September dry and bright, perfect blue mold control was obtained by maintaining night temperatures above 70°. The following year, September was very cloudy and sporulation and disease development proceeded freely at night temperatures of 70° to 75°. In midwinter in the greenhouse, the disease is very active at minimum temperatures of 70° to 80°. The statement that maintaining a minimum temperature above 70° will stop sporulation and control blue mold appears to be consistently true under usual plant-bed conditions in the spring, but it is not true in the greenhouse in midwinter.

A further illustration of the effect of the physiology of the plant on the temperature relations of the disease is the situation in New England, where tobacco grown under artificial shade sometimes is damaged severely by blue mold while the field crop grown without shade is at most very slightly affected.

It is of interest to note that temperatures during the day affect spore production the following night. It is usually considered that night temperatures of 55° to 60° F. favor sporulation, but table 2 shows that an alternation of higher and lower temperatures was much more favorable for sporulation than a uniform lower temperature.

It should be pointed out that in most of these studies temperatures were kept fairly constant, whereas under actual plant-bed conditions temperatures usually fluctuate constantly and vary widely. The fact that a maximum of 77° F. and a minimum of 60° were more favorable for sporulation than a constant temperature suggests the possibility that more frequent and wider fluctuations might have still greater influence and that such variation might have an important bearing on spore germination, infection, and subsequent disease development. Since the optimum temperature for germination of spores collected in these experiments varied from 35° to 79°, it is evident that all the factors governing viable spore production and germination are not yet understood. In the controlled experiments here reported sporulation did not occur at temperatures below 50°, yet in commercial plant beds in Georgia sporulation has occurred freely on mornings when the plant-bed cover was coated with frost and temperatures inside the bed remained below 50° all night and well into the forenoon.

Finally, it may be noted that disease development in these tests proceeded most rapidly when night temperatures remained between 55° and 62° F., but this maximum rate of development usually was confined to a few days near the end of the epidemic. When blue mold is very destructive in Georgia most of the damage occurs during long, cool periods with night temperatures between 30° and 50° and with wide ranges between maximum and minimum temperature. Blue mold has been observed to remain active during the coldest periods tolerated by tobacco plants. Disease activity is slower during such periods, but the total damage is very great.

These studies have made it possible to operate a blue mold resistance breeding program without interruption throughout the summer. To do this a large insulated chamber was constructed and adjusted to give a day temperature of 75° F. and a night temperature of 60°.

Light was provided by fluorescent tubes. Plants were inoculated and held under these conditions for 96 hours. They were then moved to a bed located in a shaded, well-protected area, with muslin covers to provide additional shade as required. Under these conditions blue mold development was uniform and severe during the hottest weather of August. Luxuriant sporulation took place the sixth and seventh mornings after inoculation, and 14 days after inoculation 70 to 95 percent of the plants were dead. In a few days more, mortality was frequently 98 to 100 percent. This serves to emphasize again the fact that, although blue mold is a cool-weather disease, some phases of its development are greatly favored by moderately high temperatures provided light is controlled.

One of the numerous puzzling problems with respect to blue mold has been the report from Bathurst, Australia (7), of effective blue mold control for 12 consecutive years by growing plants in flue-heated beds. This control was attributed to the maintenance of temperatures above 45° F. Hill and Allan (6) later found that this treatment was ineffective, as would be expected, since the disease is recognized to be most active at minimum temperatures between 40° and 60°. However, in the Bathurst report reference is made to temperatures as high as 110°, and it is stated that even 125° to 130° caused no harm if the plants were not tender. The writers' studies have shown that excellent blue mold control can be obtained with exposure for 4 hours per week at 110° and that even 2 hours per week is rather effective. In view of the potency of high-temperature treatment, it appears that at Bathurst, where the method of heating permitted wide fluctuations, the actual blue mold control achieved was the result of occasional high temperatures and not of the maintenance of temperatures above 45°.

The effectiveness of high temperature in destroying the fungus was not modified by the condition of the host plant. However, the ability of the plant to withstand high-temperature treatment was much affected by the succulence of the tissues. Very tender plants can be severely injured by treatment at 110° to 115° F. High-temperature treatment worked especially well in greenhouses, where the large volume of air and the comparative ease of temperature regulation make blue mold control by this method quite simple.

In general, temperature control of blue mold in the plant bed, either by all-night treatment with temperatures held above 70° F. or by short treatments at 110°, is not likely to be used widely because gas and spray treatments that are available are effective and less expensive. However, a shortage of materials for gas or spray treatment might well result in the use of heat treatment for blue mold control.

SUMMARY

The spores of *Peronospora tabacina* are produced in the early morning and die within a few hours unless infection occurs.

Viability of spores was affected by the temperature conditions both at the time of spore formation and at germination.

Sporulation was most abundant with a night temperature of 60° F. preceded by a higher day temperature.

Some collections of spores germinated best at cool temperatures (35°–50° F.) and other collections at warm temperatures (64°–79°); hence a single optimum temperature for spore germination does not exist.

Leaf infection was favored by temperatures of 64° to 75° F., and germination and infection were both inhibited by temperatures above 85°.

Almost no sporulation occurred in tobacco plant beds with minimum night temperatures of 70° F. and above. Maintenance of such temperatures by artificial heating gave consistent and highly effective control of blue mold.

Plants grown with reduced light, as the result of heavy shading or midwinter conditions in the greenhouse, sporulated freely with minimum temperatures as high as 80° F.; hence, under such conditions, temperature control based on inhibition of sporulation was not practicable.

The lethal effect of temperatures above 85° F. on the fungus was not modified by the condition of the host plant. Exposure to 110° for 4 hours per week controlled blue mold effectively in plants that between treatments were constantly exposed to reinfection. In the greenhouse, exposure of the plants for 5 hours per week to a temperature of 105° to 110°, using heat from the sun, gave complete control of blue mold.

Heat treatment for the control of blue mold was generally more expensive than either spray or gas treatment.

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EVALUATING FLEECE QUALITY OF NAVAJO SHEEP FROM SMALL SAMPLES[1]

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INTRODUCTION

Navajo sheep produce a mixed type of wool. The fleece has an undercoat of true wool fibers and a protective outercoat of long, coarse, hairlike fibers. In common with other wools of this type, the fleece also contains varying quantities of kemp and other medullated fibers.

Kemp is a distinct type of fiber that differs both in form and manner of growth from other medullated fibers of the undercoat and outercoat and can usually be detected by visual inspection of the fleece. Macroscopically the fibers are typically short, coarse, straight, or waved (uniplanar) with a long, tapering tip and an opaque appearance. When examined microscopically, kemp is found to consist largely of medullae, which are built up of superimposed cells that usually contain air. Duerden (1)[2] has shown that the inclusion of air in the medullae is responsible for the paleness or off color of the kemp in dyed wool. Kemp fibers are shed periodically and replaced by new growth, a characteristic that is not associated with other types of medullated fibers found in the fleeces of Navajo sheep.

Medullation is often considered a basis for distinguishing hair from wool, but it is not a positive criterion. In Navajo fleeces the medullated fibers are found principally in the coarse, hairy outercoat, but small numbers of the undercoat or true wool fibers frequently have a fine medullary canal. The medullae correspond to the three types described and illustrated by Wilson (9) and Von Bergen and Mauersberger (8) as fragmental, interrupted, or continuous, and vary in size from approximately 10 to 60 percent of the cross-sectional area of the fiber.

Outercoat, kemp, and other medullated fibers are the dominant factors that influence the economic and utility values of Navajo wool. Grandstaff (4) has shown that the suitability of the wool for hand weaving varies in direct relation to the quantities of kemp and hair fibers present in the fleece. In the tests made by him, rugs woven from Navajo wool containing fewer than 1 percent of kemp fibers and 10 percent of outercoat fibers greater than 40 microns in diameter were of the highest quality. Kemp in amounts of 3 to 5 percent interfered with the spinning of uniform yarn and resulted in rugs of

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coarse, harsh texture. Rugs made from wool containing 15 to 25 percent of outercoat fibers were of coarse, hairy texture and inferior quality.

The quantities of outercoat, kemp, and other medullated fibers vary considerably between regions of individual fleeces, and to a much greater extent between the fleeces of different sheep produced under similar environmental conditions. Hence, these variations are largely if not wholly due to the genetic composition of the sheep.

Progress in fleece improvement, through selective breeding of the sheep, depends on a reliable system of fleece sampling and analysis. The objectives of this study were to measure the distribution of outercoat, kemp, and other medullated fibers in the fleeces; to determine the minimum size of samples required for laboratory analysis; and to select certain definite anatomical areas which, individually or combined, provide the best index of fleece quality.

MATERIALS AND METHODS

At weaning time in the fall of 1939, 24 Navajo ewe and ram lambs were selected at random for a fleece-sampling test at the Southwestern Range and Sheep Breeding Laboratory, Fort Wingate, N. Mex. Preparatory to sampling, each lamb was placed on a table, and the neck, shoulder, side, and thigh regions were blocked out on the left half of the body with marking chalk. Three lines were so drawn as to divide these 4 fleece regions equally. The belly region was also divided into 4 quarters. Samples of wool about the size of 3 fingers were taken with an electric clipper from the 44 positions indicated in figure 1,
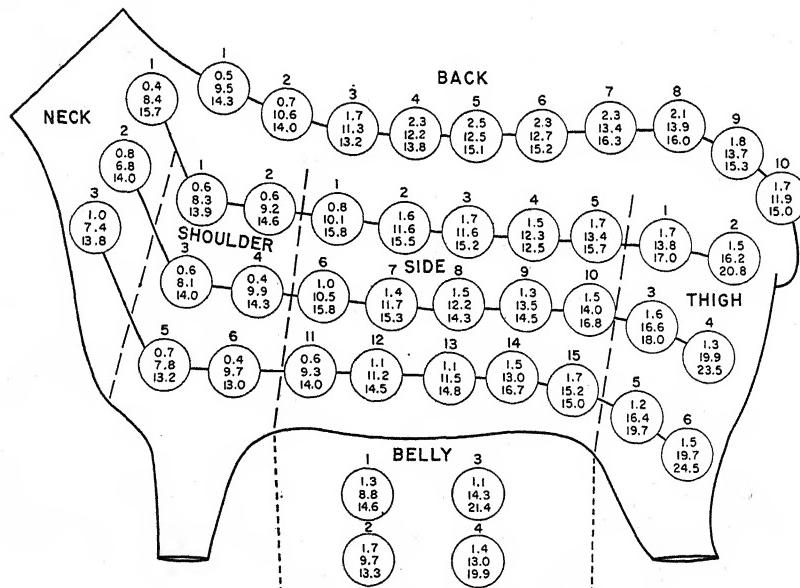
FIGURE 1. The location of positions sampled in each of the 6 regions of the fleece is indicated by numbered circles. Data from top to bottom within the circles represent the average numbers of kemp, other medullated fibers, and outercoat fibers, respectively, per 100 total fibers, observed for each position.

thus making a total of 1,056 samples for the 24 lambs. Distribution of the 44 sample positions was as follows: 10 were located at regular intervals along the backbone from the point of the withers to the tailhead, 10 along each of the 3 lines that were used to divide the left half of the body, and 1 at approximately the center of each of the 4 quarters of the belly. The total number of positions for the back was 10; neck, 3; shoulder, 6; side, 15; thigh, 6; and belly, 4.

In the laboratory each fleece sample was first divided into 11 subsamples of the same size, 10 of which were used for counts of kemp and other medullated fibers, and the eleventh for measurements of the fiber-diameter distribution. A tuft of fibers from each subsample was cross-sectioned by means of Hardy's (6) device and projected on a ground-glass field 7 by 11 inches in size. From the magnified image of the fiber cross sections, a representative portion of 100 fibers was used for counting the number of kemp and other medullated fibers in subsamples 1 to 10 and for making the diameter measurements of subsample 11. The fibers were measured by the method reported by Grandstaff and Hodde (5), and the total number of fibers more than 40 microns in diameter, exclusive of kemp, was used as the best estimate of the amount of outercoat in each subsample.

Before analyzing the data for the three types of fibers, all observations were increased by 1 for the purpose of eliminating zeros occasioned by the absence of kemp and other medullated fibers in some samples. The data were studied by analysis of variance, according to the method of Snedecor (7), and the adjustments of Ezekiel (2) for small size of samples were applied to all correlation and regression coefficients.

EXPERIMENTAL RESULTS

DISTRIBUTION OF FIBERS

Figure 1 shows the average distribution of the 3 types of fibers in the 44 positions of the fleece, based on the mean number of fibers per subsample. Variations in the number of outercoat and medullated fibers other than kemp were closely associated, whereas the amount of kemp varied independently of the other 2 types. In general, all 3 types increased pronouncedly from the neck or shoulder to the thigh. The back, however, exceeded all other regions of the fleece in quantity of kemp. The ranges in mean numbers of kemp, other medullated fibers, and outercoat fibers for the 6 regions were as follows: 0.5 to 1.8, 7.5 to 17.1, and 13.8 to 20.6, respectively. The side approximated the average of the 44 positions for the various types of fibers considered. Within fleece regions the amounts of kemp, other medullated fibers, and outercoat fibers did not vary in a systematic manner.

In order to evaluate the differences in number of the three types of fibers, the data for each type were studied separately by an analysis of variance. Analyses for kemp and other medullated fibers were based on three criteria of classification for each of the six body regions: Between sheep, between positions, and between subsamples.

Within each region, differences between sheep constituted the primary source of variation for kemp. Except for the belly region, this statement also applies to other medullated fibers. In all cases the F values were many times as great as the value required at the 1-percent level of significance. For kemp the variance due to differences

between positions was significant for the neck and highly significant for the back and side. For medullated fibers other than kemp, differences between positions represented a highly significant source of variance in all 6 regions of the fleece. The variance due to differences between the numbers of kemp fibers in 10 subsamples for each position was not significant in any fleece region, whereas for the other medullated fibers the variance between subsamples was highly significant in all regions of the fleece.

The variance due to interaction of sheep $\times$ position was considerably larger in most instances than the variance due to differences between subsamples. For kemp the F values for sheep $\times$ position were not significant except for the back and belly, whereas for medullated fibers they were highly significant for all regions of the fleece. The variances due to interactions of sheep $\times$ samples and positions $\times$ samples were also substantially greater for other medullated fibers than for kemp.

Separate analyses of the total numbers of kemp and other medullated fibers for the six fleece regions showed that the differences between regions were highly significant.

In the data on outercoat fibers differences between sheep were also a major source of variation. An analysis of the variance within and between positions and regions of the fleece showed that differences between positions were significant for the thigh and belly only, and differences between regions of the fleece were highly significant.

MINIMUM SIZE OF SAMPLES REQUIRED

From a practical standpoint the size and number of samples required for evaluating quality of whole fleeces are factors of primary importance. The usual practice at the Southwestern Range and Sheep Breeding Laboratory has been to sample the fleece of each sheep from the middle of the side, thigh, and back, and to use a sample of 100 fibers from each of the 3 positions for evaluating average amounts of outercoat, kemp, and other medullated fibers.

To determine the minimum size of sample required for measuring the amounts of kemp and other medullated fibers within fleece positions, data for the 10 subsamples of position 1 in each fleece region were studied by analysis of variance. For this purpose the data were grouped so that the variance of the first subsample was compared with that of the first 3, first 5, and all 10 subsamples for each of the respective positions. It was found that the variance within samples of 100, 300, 500, and 1,000 fibers was much greater than the variance between the 4 different-sized samples for each position. It appeared, however, that the results of this analysis might have been somewhat biased, since subsample 1 was also a part of the 3, 5, and 10 subsamples with which it was compared. To eliminate the possibility of bias, the variance of subsample 1 was compared with that of the next 3 (subsamples 2 to 4, inclusive), next 5 (subsamples 2 to 6), and last 9 (subsamples 2 to 10). Here again the variance within the different-sized samples was found to be significantly greater than the variance between samples. These results indicated that a sample of 100 fibers was as reliable as a sample of 1,000 fibers for detecting real differences between sheep in number of kemp and other medullated

fibers in each fleece position studied. On the basis of these facts, it appeared safe to assume that a sample of 100 fibers would also provide a reliable estimate of the amount of outercoat in each fleece position. It was realized, however, that a sample of more than 100 fibers might be required for evaluating differences between sheep when the fleeces are more uniform than those in the present study.

AREAS PROVIDING BEST INDEX OF FLEECE QUALITY

As a basis for selecting a representative position within each fleece region, the numbers of kemp and other medullated fibers in the first subsample from each position were studied by analysis of variance. The results showed that in all regions except the belly, the variance within positions was significantly greater than the variance between positions, when the 24 sheep were considered as a unit. Consequently, it appeared that the neck, shoulder, side, thigh, and back could each be evaluated from a single sample of 100 fibers taken from any one of the several positions sampled within each of these regions. The following positions were therefore chosen arbitrarily as representative of their respective regions: Neck, 2; shoulder, 3; side, 7; thigh, 3; back, 5 (fig. 1). The positions indicated for the side, thigh, and back are similar to those ordinarily used when sampling the fleeces for the 3 types of fibers. Since the belly represented a small and relatively unimportant part of the fleece, position 1 was arbitrarily chosen for this region even though the analysis of variance showed that there were significant differences between the belly positions.

With the use of 6 selected positions, the data were analyzed by means of simple and multiple correlations and regressions. These positions were correlated with one another and with the remaining 38 positions for numbers of the 3 types of fibers. In this study the 38 positions represented the best measure of the whole fleece. From a comparison of the interposition correlations for each type of fiber it was impossible to show any consistent relationship between the fiber content of adjacent and more distant parts of the fleece. In the kemp fibers, for instance, the contiguous regions, neck-shoulder, shoulder, side, side-thigh, and neck-thigh, had correlations of $+0.79$, $+0.46$, $+0.48$, and $+0.63$, respectively. When all the correlations were considered, however, 11 of those for kemp, 10 for other medullated fibers, and 12 for outercoat fibers were significant or highly significant. The correlations between the belly sample and the other 5 positions tended to be low and statistically insignificant, especially for outercoat fibers and medullated fibers except kemp. On the other hand, the correlations for the side and thigh with each of the remaining 4 selected positions exceeded the expected P value of 0.404 required at the 5-percent level of significance and were of about the same magnitude for outercoat, kemp, and other medullated fibers.

Table 1 shows that the neck, shoulder, side, thigh, and back have highly significant positive correlations with the other 38 positions for the 3 types of fibers. The correlation coefficients for these 5 positions ranged from $+0.66$ to $+0.88$ for kemp, $+0.62$ to $+0.89$ for other medullated fibers, and $+0.72$ to $+0.88$ for outercoat fibers. Differences between the correlation coefficients for each of the 3 kinds of

fibers were tested by means of Fisher's (*3*) z transformation and found to be statistically insignificant. The correlation for the thigh was highest for kemp, whereas that for the side was highest for other medullated fibers and outercoat fibers. The correlations for the belly and the 38 unselected positions were consistently low, but they were significant in all cases.

TABLE 1.—*Relation between average numbers of the 3 types of fibers in samples from 38 fleece positions (Y) and 1 selected position (X) from each of the 6 regions*

KEMP FIBERS

Fleece region and position [1]	Adjusted correlation coefficients [2]	Regression coefficients [3]		Standard errors of estimate	Reduction from standard deviation of 38 positions to standard error of estimate
		a	*by.x*		
					Percent
Neck, 2	0.67	0.92	0.94	1.45	26
Shoulder, 3	.66	.65	1.21	1.48	25
Side, 7	.75	.91	.72	1.30	34
Thigh, 3	.88	.81	.60	.94	52
Back, 5	.76	.69	.50	1.27	36
Belly, 1	.50	1.34	.53	1.70	13

OTHER MEDULLATED FIBERS

Neck, 2	0.62	7.88	0.73	4.65	21
Shoulder, 3	.78	6.42	.76	3.70	37
Side, 7	.89	3.20	.76	2.63	55
Thigh, 3	.78	4.37	.52	3.70	37
Back, 5	.75	2.53	.84	3.94	33
Belly, 1	.49	7.50	.55	5.15	13

OUTERCOAT FIBERS

Neck, 2	0.79	7.52	0.60	3.78	38
Shoulder, 3	.74	8.57	.52	4.09	33
Side, 7	88	6.08	.64	2.87	53
Thigh, 3	.72	6.23	.53	4.24	31
Back, 5	.73	4.68	.74	4.20	31
Belly, 1	.51	10.72	.35	5.26	14

[1] Location of each position shown in figure 1.
[2] For 22 degrees of freedom the 5-percent level of significance is 0.404, and the 1-percent level of significance is 0.515. All correlation coefficients are positive.
[3] *a* and *b* are the coefficients necessary to the expression of any linear regression. All regression coefficients are positive.

The regression coefficients, *by.x*, were used to establish the predictive value of each of the 6 selected positions in estimating the average fiber content of the 38 positions. The information obtained by solving the regression equations is also given in table 1. The thigh sample had the lowest standard error of estimate for kemp fibers and accounted for 52 percent of the variance found in the 38 positions. For the other medullated fibers and the outercoat fibers the side sample had the lowest standard error of estimate and reduced the variance of the 38 positions by 55 and 53 percent, respectively.

From the data presented in table 1, it is apparent that some combination of two or more positions should be used in evaluating the fleece. The 15 possible pairs of selected positions were correlated with the remaining 38 positions in order to determine which combination would

yield the most information. All multiple correlation coefficients for the 3 types of fibers exceeded the P value of 0.596 required at the 1-percent level of significance. The coefficients ranged from +0.68 to +0.95 for kemp, +0.72 to +0.95 for other medullated fibers, and +0.79 to +0.93 for outercoat fibers. The combination of the side and thigh yielded the highest correlation for kemp and other medullated fibers (table 2) and next to the highest for outercoat fibers. Moreover, the coefficients of multiple determination showed that 77 to 90 percent of the variation in numbers of kemp fibers in the 38 positions could be accounted for when the thigh sample was one of the two positions considered. In the other medullated fibers and the outercoat fibers, the use of the side sample as one of the two positions accounted for 70 to 91 percent, and 79 to 87 percent, respectively, of the variance of these types of fibers in the 38 positions.

TABLE 2.—*Relation between the average numbers of the 3 types of fibers in samples from 38 fleece positions (Y) and 1 selected position from each of the side and thigh* [1]

Type of fiber	Adjusted multiple correlation coefficients [2]	Net regression coefficients [3]			Standard error of estimate	Reduction from standard deviation of 38 positions to standard error of estimate
		a	$by.x_1$	$by.x_2$		
						Percent
Kemp	0.95	0.32	0.40	0.45	0.63	68
Other medullated	.95	1.52	.56	.24	1.83	72
Outercoat	.92	3.76	.50	.25	2.38	61

[1] Selected positions were side 7 (X_1) and thigh 3 (X_2).
[2] For 21 degrees of freedom the 1-percent level of significance is 0.596. All correlation coefficients are positive.
[3] a and b are the coefficients necessary to the expression of any linear regression. All regression coefficients are positive.

The combination of side and thigh yielded the best estimate of the 38 positions, when all 3 fiber types were considered in evaluating the fleece. For kemp this combination accounted for 68 percent, for other medullated fibers 72 percent, and for outercoat fibers 61 percent, of the variance found in the 38 positions. In the outercoat fibers, the neck and side accounted for 3 percent more of the total variance of the 38 positions than the side and thigh. This small gain for outercoat fibers did not seem to justify the use of the neck sample, since it proved to be less reliable than the thigh as an index of the amounts of kemp and other medullated fibers in the 38 positions.

The net regression coefficients for the side and thigh combination were positive for all 3 types of fibers studied. This means that for every unit increase in fiber content of the side and thigh, the amount of change in the 38 positions would be the sum of the increases for the 2 positions. Within the limits of this study, however, the coefficients of determination showed that for kemp 23 percent, for other medullated fibers 31 percent, and for outercoat fibers 29 percent, of the variance were common to both the side and thigh. The actual increase in fiber content of the 38 positions, therefore, would be less

in some instances than that determined mathematically from the multiple regression equations.

Since the fleeces have been sampled heretofore from the side, thigh, and back, it was considered advisable to compare these 3 positions, as well as the 6 positions representing all regions of the fleece, with the average of the 38 positions. The multiple correlations obtained for these 2 sets of comparisons were as follows: Kemp, $+0.95$ and $+0.95$; other medullated fibers, $+0.97$ and $+0.97$; and outercoat fibers, $+0.93$ and $+0.95$, respectively. Comparison of these correlation coefficients with those obtained for 2 positions (side and thigh) and the 38 positions showed no differences for kemp and only slight increases for other medullated fibers and outercoat fibers.

The use of the side, thigh, and back accounted for only 2 percent more of the total variance for outercoat fibers and 3 percent for medullated fibers other than kemp, than the use of the side and thigh. The use of the 6 positions reduced the total variance for outercoat and medullated fibers other than kemp by 5 percent in each case, but only 1 percent for kemp, as compared with the use of only the side and thigh. Thus it appears that little is gained by using more than the side and thigh as a basis for estimating the amounts of outercoat, kemp, and other medullated fibers in the fleece.

APPLICATION OF RESULTS

In applying the prediction equations for each of the 3 variables (kemp, other medullated fibers, and outercoat fibers), the average for 38 positions (Y) may be estimated from the values obtained for the side (X_1) and thigh (X_2) by the use of the formula $\overline{Y} = a + by \cdot x_1 X_1 + by \cdot x_2 X_2$. The a and b values given in table 2 for the 38 positions, position 7 of the side, and position 3 of the thigh are applied in solving this equation. Thus the predicted number of kemp fibers for the 38 positions, from observed values of 2 kemp for the side and 6 kemp for the thigh, would be calculated as follows: $0.32 + (0.40 \times 2) + (0.45 \times 6) = 3.82 \pm 0.63$.

In applying the prediction equation to single fleeces, it should be remembered that the standard errors of estimate define the range within which approximately two-thirds of the predicted $\overline{Y}$ values will fall. Consequently, the estimated totals of the three types of fibers for about one-third of the fleeces may be either larger or smaller than their true values. Nevertheless, the limits of accuracy inherent in the method of fleece sampling and evaluation presented herein are considered to be well within the bounds of control that can be accomplished in actual breeding practice with Navajo sheep.

The predicted fleece values for the three types of fibers at weaning age will be useful in conducting progeny tests of the rams used each year. Errors made in evaluating individual fleeces will tend to compensate each other when the data are combined and analyzed for progeny groups. Consequently, it should be possible to detect fleece differences between progeny groups with a high degree of certainty.

Although the purpose of this study is to illustrate a method of evaluating the fleeces of Navajo sheep from small samples, it should also be useful in conducting fleece-sampling tests with other types of sheep.

SUMMARY

A fleece-sampling test was conducted at the Southwestern Range and Sheep Breeding Laboratory, Fort Wingate, N. Mex., to develop a practical and reliable system of evaluating the amounts of outercoat, kemp, and other medullated fibers in the fleeces of Navajo sheep at weaning age. The work was conducted in the fall of 1939 with 24 ewe and ram lambs. Wool samples were taken from 44 positions on the back, belly, neck, shoulder, side, and thigh of the left half of the body of each animal.

Throughout the fleece, variations in the amount of outercoat fibers and medullated fibers other than kemp were closely associated, whereas kemp varied independently of the other two types.

By means of analyses of variance the following significant facts were established: (1) Within fleece regions the differences between sheep were the major source of variance in all but one instance; (2) the variance between fleece regions was many times as large as the variance within regions; (3) when the 24 sheep were considered as a unit, a sample of 100 fibers proved to be as reliable as 1,000 fibers for detecting significant differences between sheep; (4) the variance within positions, based on samples of 100 fibers, was significantly greater than the variance between positions, except for the belly region.

Comparison of 1 selected position for each of the 6 fleece regions with the average of the remaining 38 positions yielded significant or highly significant correlations, the thigh being highest for kemp and the side highest for other medullated fibers and outercoat fibers.

For evaluating the fleeces, combinations of 2, 3, and 6 positions were compared with the average of 38 positions. All multiple correlation coefficients were highly significant. Of the various combinations of 2 positions, the side and thigh ranked highest for kemp and other medullated fibers and next to the highest for outercoat fibers. These two positions reduced the total variance 68, 72, and 61 percent, respectively, for the 3 types of fibers. Combinations of 3 and of 6 positions proved to be slightly superior to the combination of side and thigh for evaluating outercoat fibers and medullated fibers other than kemp. From a practical standpoint, however, the results did not justify the use of more than these 2 positions, particularly when the 3 types of fibers were considered.

The 3 regression equations for 38 positions on the side and thigh provide the necessary information for use in estimating the average amounts of the 3 types of fibers in the fleece from data for the 2 selected positions.

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MYCOSPHAERELLA BLACK ROT OF CUCURBITS [1]

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INTRODUCTION

New York, N. Y., importers of cucumbers (*Cucumis sativus* L.) have in recent years suffered heavy losses from decay in boat shipments from Puerto Rico and Cuba.[2] Mycosphaerella black rot, caused by *Mycosphaerella citrullina* (C. O. Sm.) Gross. (*12*),[3] was found to account for most of the spoilage. Investigation of this disease was therefore begun at the Market Pathology Laboratory of the United States Department of Agriculture at New York in 1938 and was continued at irregular intervals through 1942. The study also included observations on the decay of other cucurbits of both foreign and domestic origin.

DISTRIBUTION AND ECONOMIC IMPORTANCE

Many investigators have noted the occurrence of cucurbit diseases caused by *Mycosphaerella citrullina*. Symptoms may include spotting of the leaves; production of cankers on stems, petioles, and fruitstalks; decay of the stem; wilt of the vine; and rotting of the fruit. A summary of many such reports is given in table 1.

On the market Orton and Meier (*22*) found *M. citrullina* causing both stem-end and blossom-end rots of Florida and Georgia watermelons at New York. Market observations of a mycosphaerella black rot of cucumber fruits were first made at New York by F. C. Meier.[4] These were followed by more complete transit and market studies by Meier, Drechsler, and Eddy (*18*).

Brief reports based on the studies here summarized have already appeared (*4, p. 47*).[5][6]

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[1] Received for publication September 9, 1943.

[2] Processed reports issued by the Agricultural Marketing Service, U. S. Department of Agriculture, show that from 1938 to 1941, inclusive, an average of 3,061 carlots of cucumbers were unloaded annually at New York, N. Y., of which 226 carlots (7.4 percent) were received from Puerto Rico and Cuba (Isle of Pines). Most of these imports arrived during the 4 months of December to March and constituted 80.7 percent of all cucumbers received annually at New York during that period.

[3] Italic numbers in parentheses refer to Literature Cited, p. 211.

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TABLE 1.—*Cucurbit diseases caused by Mycosphaerella citrullina*

Suscept	Disease reported	Location	Source of report
Cucumis sativus (cucumber).	Mycosphaerella wilt or gummy stem blight of vines; black rot of fruits.	Florida, Massachusetts, New Jersey, New York, and Trinidad, British West Indies.	Weber (*36*); Boyd (*5, p. 207*); Wardlaw, Leonard, and Baker (*35*).[1][2]
Cucumis melo L. (muskmelon).	Stem canker, mycosphaerella wilt, or gummy stem blight of vines; black rot of fruits.	Florida, Massachusetts, New York, and Japan.	Hemmi (*13*); Sherbakoff (*30*); Boyd (*5, p. 207*).[2][3][4]
Citrullus vulgaris Schrad.(watermelon).	Mycosphaerella wilt or gummy stem blight of vines; decay of fruits.	Alabama, Arizona, Florida, Georgia, Louisiana, Massachusetts, Mississippi, Missouri, New York, South Carolina, Virginia, and Puerto Rico.	Sherbakoff (*30, 31*); Walker (*33, p. 88*); Walker and Weber (*34*); Orton (*21*); Orton and Meier (*22*).[2][5]
Cucurbita maxima Duchesne (winter squash).	Gummy stem blight of vines; black rot of fruits.	Maine, Massachusetts, and Michigan.	Kauffman (*14*); Boyd (*5, p. 207*).[2][6]
Cucurbita pepo L. (summer squash, pumpkin, vegetable marrow and warty gourd).	Wilt of vines; black rot of fruits.	Massachusetts, Michigan, New Jersey, New York, and Union of Soviet Socialist Republics.	Boyd (*5, p. 207*); Rodighin (*26*).[2][7]
Cucurbita moschata Duchesne (Cushaw squash).	Vine wilt; fruit rot____	Massachusetts_____	Boyd (*5, p. 207*).
Sechium edule (Jacq.) Swartz (chayote).	Not specified_____	Florida_____	(¹).

[1] See also pp. 251 and 252 of reference in footnote 4, p. 193.
[2] Also various numbers of Plant Disease Reporter and Plant Disease Reporter Supplement issued by the Bureau of Plant Industry. [Processed.]
[3] CHUPP, C. MYCOSPHAERELLA WILT OF MELONS IN NEW YORK. U. S. Bur. Plant Indus., Plant Dis. Rptr. 16: 143–144, 1932. [Processed.]
[4] In a letter of October 4, 1938, to the author, Dr. Charles Chupp reported again finding muskmelon fruits affected with an advanced stage of mycosphaerella black rot.
[5] Numerous annual reports of Florida Agricultural Experiment Station.
[6] Numerous annual reports of the Massachusetts Agricultural Experiment Station.
[7] See also footnote 8, p. 207.

DECAY OF CUCUMBERS ON THE MARKET

Decay was prevalent in many Puerto Rican shipments of cucumbers received during each of the four winter seasons 1938–39 to 1941–42, inclusive. Mycosphaerella black rot was regularly the most important type of decay found. In many shipments it alone was responsible for all the spoilage noted.

Losses from black rot were extremely variable in both distribution and amount. In many cargoes practically none occurred. In others the crates of many brands had from a few to practically all of their contents affected with this decay.

The representative of one large group of shippers has estimated that damage from decay was reflected to an important extent in the lower sale price received for approximately 31 percent of 20,000 crates imported during the 1939–40 season. Most of the decay was mycosphaerella black rot. This decay was likewise the most important one found by Federal food-products inspectors during their examination of Puerto Rican lots totaling over 23,000 crates (table 2), in which approximately 23 percent of the fruits were spoiled.

Imports from Cuba (table 2) were found affected with black rot throughout the 4-year period, although losses were not so great as those in imports from Puerto Rico.

Only once was mycosphaerella black rot observed on domestic cucumbers; the diseased fruits were found in a rail shipment from Georgia that arrived at New York during the summer of 1938.

TABLE 2.—*Occurrence of mycosphaerella black rot on cucumbers at the New York market* [1]

Origin	Season	Inspec-tions made	Crates inspected	Fruits decayed [2]
		Number	*Number*	*Percent*
Puerto Rico	1938–39	16	2,920	11.0
	1939–40	11	2,459	6.2
	1940–41	21	9,336	16.4
	1941–42	29	8,783	38.9
Cuba [3]	1939–40	6	1,584	8.5
	1941–42	2	486	9.6

[1] Data based on observations made by inspectors of the Agricultural Marketing Administration, U. S. Department of Agriculture.
[2] Mostly mycosphaerella black rot.
[3] Black rot was observed in 21 other shipments during the 4 seasons. Observations made (at least in part) on fruit collected by inspectors of the Bureau of Entomology and Plant Quarantine, United States Department of Agriculture.

DECAY OF OTHER CUCURBITS ON THE MARKET

Though of commercial importance only on Puerto Rican and Cuban cucumbers, mycosphaerella black rot was noted on several other domestic and imported cucurbits. The results of these observations are summarized in table 3.

Only limited data were obtained on watermelon from the Southern States or on domestic squash. Consequently it cannot be stated definitely to what extent black rot is found on these cucurbits.

Extensive observations on cantaloups and on Honey Dew, Honey Ball, and related melons have been made continuously at this market for a number of years. In no instance has mycosphaerella black rot been found on any of these domestic muskmelons. Nor has it ever been found during the course of frequent observations of cantaloups imported from Mexico, or on Honey Dew melons from Chile and Argentina. The occurrence of black rot on the muskmelon fruit presumably originating in Venezuela (table 3) is thus of unusual interest.

TABLE 3.—*Occurrence of mycosphaerella black rot on cucurbits other than cucumbers at the New York market*

Suscept	Origin	Notes
Watermelon	Southern States	Few observations made; black rot not found.
	Cuba	Black rot found affecting a few melons in shipment received in mid-September 1940.
Hubbard squash (*Cucurbita maxima*).	Massachusetts	Noted on several fruits sent from Boston, Mass., market by Agricultural Marketing Administration inspector.
	Virginia	Carlot with 20 percent of the fruit affected by black rot, mid-December 1940.
Yellow Crookneck squash (*Cucurbita pepo*).	Cuba	*Mycosphaerella citrullina* found fruiting on stem attachments of several fruits, February 1940.
Chayote	Jamaica, British West Indies; Cuba.	Black rot of fruits observed on numerous occasions during January and February 1940 mostly scattered in small lots.[1]
	Puerto Rico	Black rot of fruits noted on several occasions.
Balsam pear (*Momordica charantia* L.).	Cuba	Black rot of fruits noted on 5 occasions during January and February 1940.[1]
Chinese preserving melon (*Benincasa hispida* (Thunb.) Cogn.).	do	Black rot of fruit noted January 1937.[1]
Muskmelon	Venezuela (?)	Single fruit with black rot found in ship's stores of a vessel arriving from Venezuela.[1]

[1] Observations made (at least in part) on fruit collected by inspectors of the Bureau of Entomology and Plant Quarantine.

SYMPTOMS

The first symptoms of the disease on cucumber fruits are small, dark, greasy or water-soaked spots (fig. 1, *A* and *B*), which may occur anywhere on the surface of the fruits. These spots may be roughly
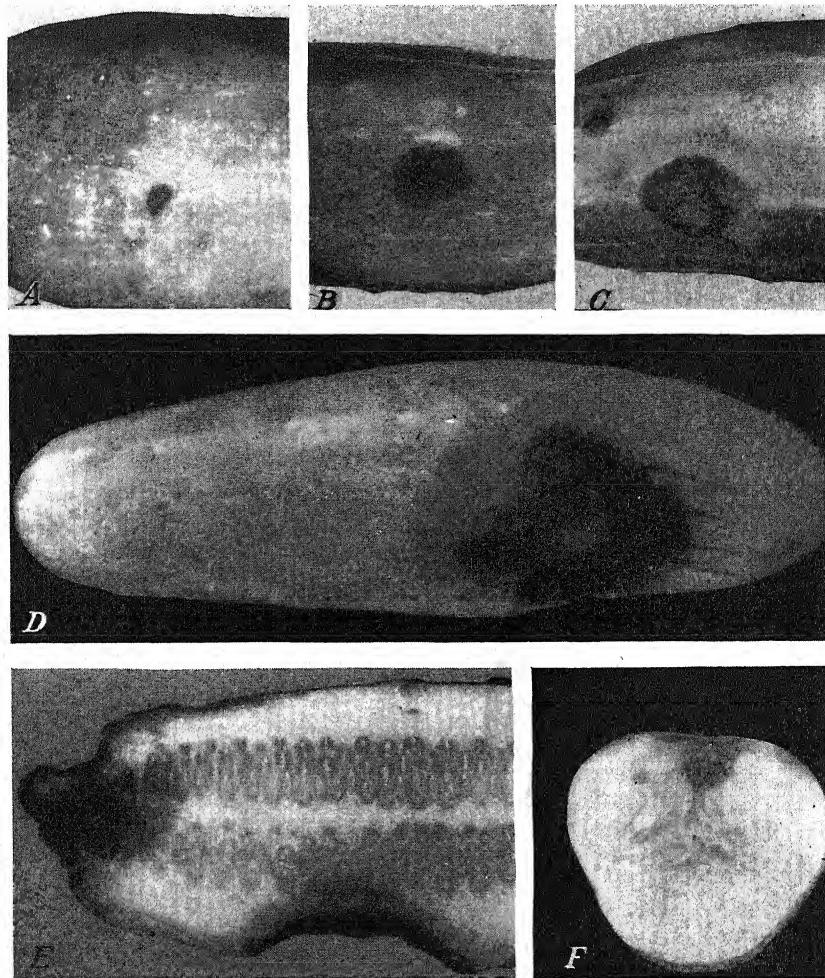
FIGURE 1.—Mycosphaerella black rot of cucumber: *A–C*, Early stages, with gummy exudate shown in *C*; *D*, advanced stage, showing shriveling, wrinkling, and blackening; *E*, longitudinal section through lesion; *F*, cross section through lesion.

circular with well-defined margins, or they may be of irregular shape with indefinite margins. Frequently a gummy exudate develops at the center of the lesion and dries, forming a firm deposit (fig. 1, *C*). This, however, is not an invariable symptom.

On dark-green cucumbers that may be described as "grass green"[7] or "spinach green" and on lighter green fruits that are "parrot green"

[7] Color designations in quotation marks are according to Ridgway (*25*).

and "courge green" to "Biscay green," the lesions in early stages are variously "yellowish olive," "dark greenish olive," "deep olive," or "Lincoln green." The more advanced lesions on lighter colored or more yellowed fruits are frequently "honey yellow," "Isabella color," or "buffy citrine."

During the early stages, particularly if the color changes are not marked and the gummy exudate is prominent, the decay might be mistaken for bacterial spot, caused by *Pseudomonas lachrymans* (E. F. Sm. and Bryan) Ferraris. Larger, darker spots in which no exudate has formed may, on the other hand, resemble early lesions of bacterial soft rot, caused by *Erwinia carotovora* (Jones) Holland, in which extensive softening has not yet occurred.

Although lesions may become considerably advanced without turning dark, the most important symptom of the later stages of decay is the blackish discoloring of the affected areas (fig. 1, *D*) that first develops at the point of inoculation. Only at this time is the decay literally a black rot.

Blackening of the lesions is accompanied by a drying out of diseased tissues, with consequent shriveling and wrinkling of the cucumber. Unless secondary bacterial decay sets in, the entire fruit becomes a shriveled, blackened mummy.

Fruiting bodies (pycnidia) of the pathogen, with their exuding masses of spores (see fig. 3, *A* and *B*), constitute an important diagnostic character. Another is the mycelium of the fungus, whitish at first, which is frequently found on the surface of the lesions (see fig. 3, *B*). This develops readily under moist conditions. Thus, when many cucumbers in a crate are found with advanced lesions, the abundant mycelium becomes wet and appressed to the cucumber surface. At such times the decay may be confused with cottony leak, caused by *Pythium aphanidermatum* (Edson) Fitz.

Internal symptoms (fig. 1, *E* and *F*) are much less striking than external ones. The affected tissues are spongy soft, ranging from rather dry to fairly moist, and are frequently somewhat vacuolated. Brownish discoloration ("chamois," "buckthorn brown," and "Dresden brown") may usually be noted throughout the older areas of the lesion. The black discoloration, so conspicuous in external view, is largely confined to the rind, although an irregular blackening may occasionally be in evidence throughout portions of the diseased tissues, particularly just beneath the center of the lesion. Where infection has occurred only at the blossom end—a common occurrence on small fruits—the black, dried, decaying tip forms a conspicuous symptom.

Seen in longitudinal or cross section, the diseased tissues are irregular in outline and indefinite in extent.

No marked or specific odor is associated with the decay.

On chayote fruits the affected tissues are at first brownish. As the lesions enlarge, the surface becomes depressed, shriveled, and blackened (fig. 2, *A*). The abundant pycnidia with exuding masses of spores are a conspicuous feature. Both external and internal margins of the lesion are irregular and indefinite. The decayed tissues are brownish to black and rather shallow.

The single muskmelon fruit found with mycosphaerella black rot (fig. 2, *B*) had been infected at the blossom end. The decayed areas

were smooth and black with sharply defined borders. No fruiting bodies developed on the lesions. Internally the decayed tissues were spongy soft and black and extended well into the flesh.

The decay on Hubbard squash appeared as a large, brownish, water-soaked lesion at the blossom end. In advanced stages the rind became black and deeply wrinkled (fig. 2, *C*). Perithecia, and pycnidia with exuding spore horns, were conspicuous.
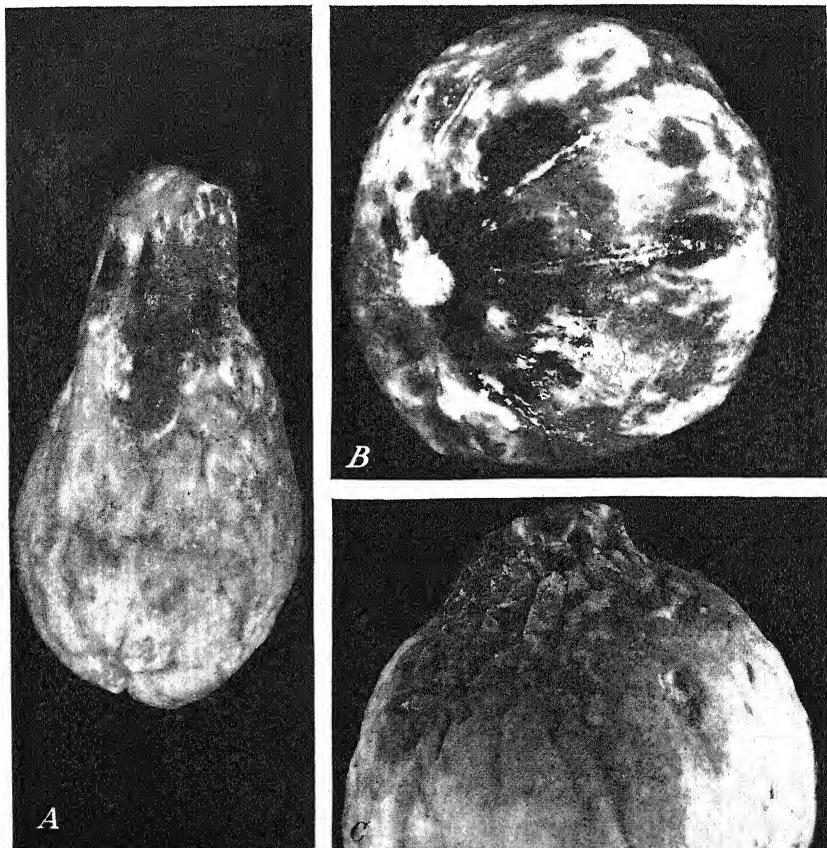
FIGURE 2.—Mycosphaerella black rot: *A*, Chayote from Jamaica, British West Indies, after 4 days at 70° to 75° F.; *B*, muskmelon from ship's stores of vessel arriving at New York, N. Y., from Venezuela; *C*, advanced lesion on Hubbard squash from Boston market.

The lesions on balsam pear were shriveled, slightly sunken, and brownish to black. The chief identifying character was the presence of the pycnidia with their conspicuous exuded spore horns.

Artificial inoculations on dishcloth gourd (*Luffa acutangula* (L.) Roxb.) yielded light-brown lesions with fairly regular but not sharply defined borders. The diseased tissues were fairly firm and slightly sunken. Here, too, the chief character for identification of the decay was the abundant pycnidial sporulation.

THE PATHOGEN

Studies were made of the pathogen in naturally occurring lesions on fruits of balsam pear, chayote, cucumber, and squash. Isolates from balsam pear, chayote, cucumber, muskmelon, squash, and watermelon were compared both in culture and in wound-inoculation lesions on cucumber fruits. Considerable variation noted in the behavior of different isolates was not found to be correlated with the types of cucurbit from which the isolates were originally obtained.

DEVELOPMENT ON CUCURBIT FRUITS

The following description of the pathogen deals primarily with its appearance on cucumber fruits in lesions that developed from both natural and artificial inoculation.

The first evidence of the pathogen is the early appearance of numerous pycnidia in the central area of the lesion. Thus mature pycnidia have been found 5 days after inoculation on cucumbers held at 70° to 75° F. At first the pycnidia are faintly brownish and entirely subepidermal. Although developed in great numbers they are at this time practically invisible to the unaided eye. Their presence is, however, readily indicated by the exuding masses of conidia that push through the broken epidermis and pile up on the surface (fig. 3, *A* and *B*). The spore masses may appear as moist, creamy, pinkish mounds or, on older, drier lesions, as firm, twisted or coiled, pinkish spore horns.

On young lesions the pycnidia have been found originating in the mesophyll just below the palisade layer and approximately 30μ to 60μ beneath the surface. More commonly they originate between the palisade cells and, by enlargement through growth, come to lie immediately beneath the epidermis.

On older lesions the abundant, dark mycelium growing throughout the rind tissues forms a semicompact layer at the surface of the cucumber fruit. The epidermis disappears, and the palisade layer becomes disorganized and is eventually destroyed. Here the pycnidia originate within the mycelium, and as they enlarge they come to lie nearly free on the surface of the lesion (fig. 3, *C, D*).

The pycnidia are nearly spherical though frequently narrowed toward the base (fig. 3, *D*), slightly longer than wide, and somewhat flattened at the apex, where the conidia are released through a single pore (fig. 3, *E*). Although light brownish at first, the pycnidia later darken and become nearly black. They vary greatly in size within a usual range in diameter of 109μ to 160μ. Two pycnidia were noted with diameters of 179μ and 207μ. All measurements were made from cucumber lesions resulting both from natural infections and from artificial inoculations with cucumber and squash isolates.

The conidia are produced in great abundance and emerge from the pycnidia in quantity, forming long, gelatinous spore horns. The release of the conidia can readily be observed under the lower power of the microscope (fig. 4, *A*).

The conidia are hyaline, either nonseptate or uniseptate, with rounded ends, and are somewhat constricted at the septa (fig. 4, *B* and *C*). Rarely does one observe a biseptate conidium. Considerable variation exists in the relative abundance of nonseptate and uniseptate spores.

There was marked uniformity in the size of conidia from different sources. The following measurements are based on spores from natural infections on balsam pear, chayote, cucumber, and squash; and from
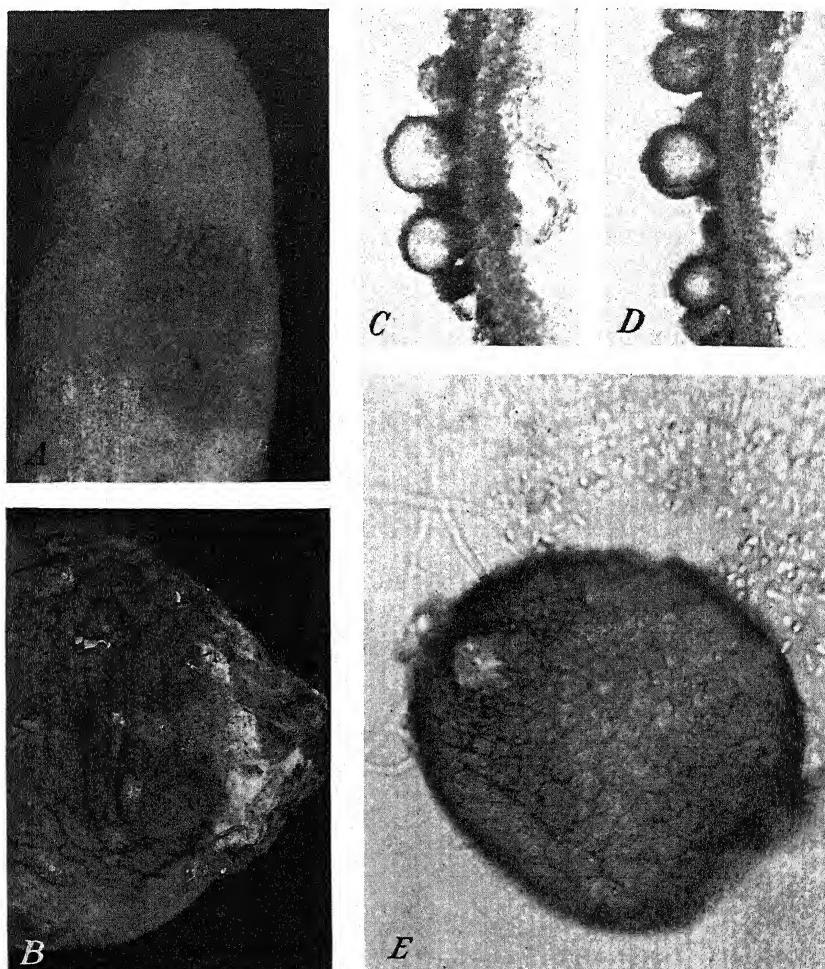
FIGURE 3.—*Mycosphaerella citrullina* on cucumber: *A*, Conidial masses first appearing on light-colored lesion; *B*, pycnidia and exuded masses of conidia found in great abundance over most of advanced lesion, with whitish mycelium developed over part of lesion; *C* and *D*, transverse freehand section through diseased rind, showing superficial pycnidia with palisade layer and portion of mesophyll beneath, × 95; *E*, single pycnidium, showing apical pore, × 430.

cucumbers artificially inoculated with isolates from cucumber, muskmelon, squash, and watermelon.

Nonseptate conidia averaged 4.0μ by 7.7μ, with a range of 2.8μ to 5.5μ by 4.6μ to 11.0μ. Approximately 85 percent of the spores fell within the limits of 3.7μ to 5.0μ by 5.5μ to 9.6μ. Uniseptate conidia averaged 4.4μ by 10.3μ, with a range of 2.8μ to 5.5μ by 7.3μ to 14.7μ. Approximately 98 percent fell within the limits of 3.7μ to 5.5μ by 8.3μ
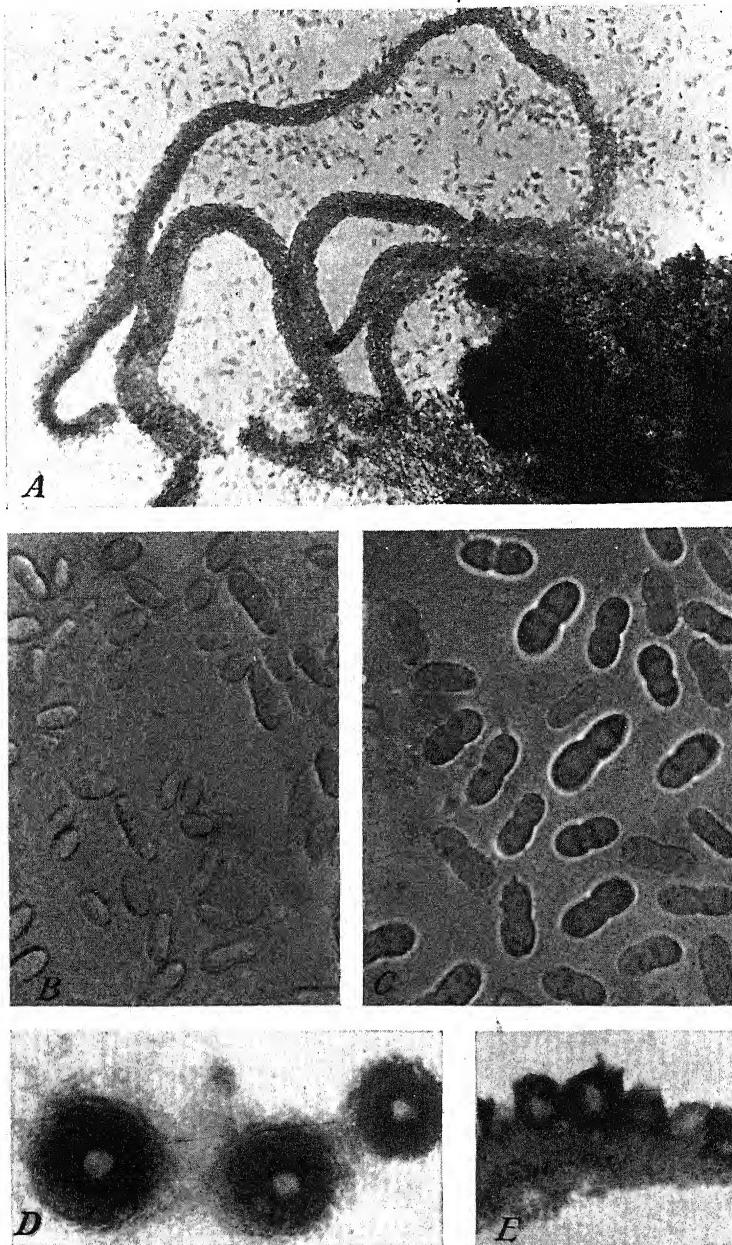
FIGURE 4.—*Mycosphaerella citrullina: A,* Two pycnidia with conidia exuded in prominent spore chains, × 95; *B,* conidia, mostly nonseptate, × 900; *C,* conidia, mostly uniseptate, × 900; *D,* scattered perithecia developed beneath the epidermis near margin of black rot lesion on Hubbard squash, × 95; *E,* transverse freehand section through diseased cucumber rind, showing superficial and nearly superficial perithecia, × 95.

to 13.8μ.   A limited number of biseptate spores averaged 4.5μ by
15.4μ, with a range of 4.1μ to 5.5μ by 12.8μ to 19.3μ.

Perithecia develop later than pycnidia and are ordinarily found in
great numbers, together with the latter, on the older, darkened areas
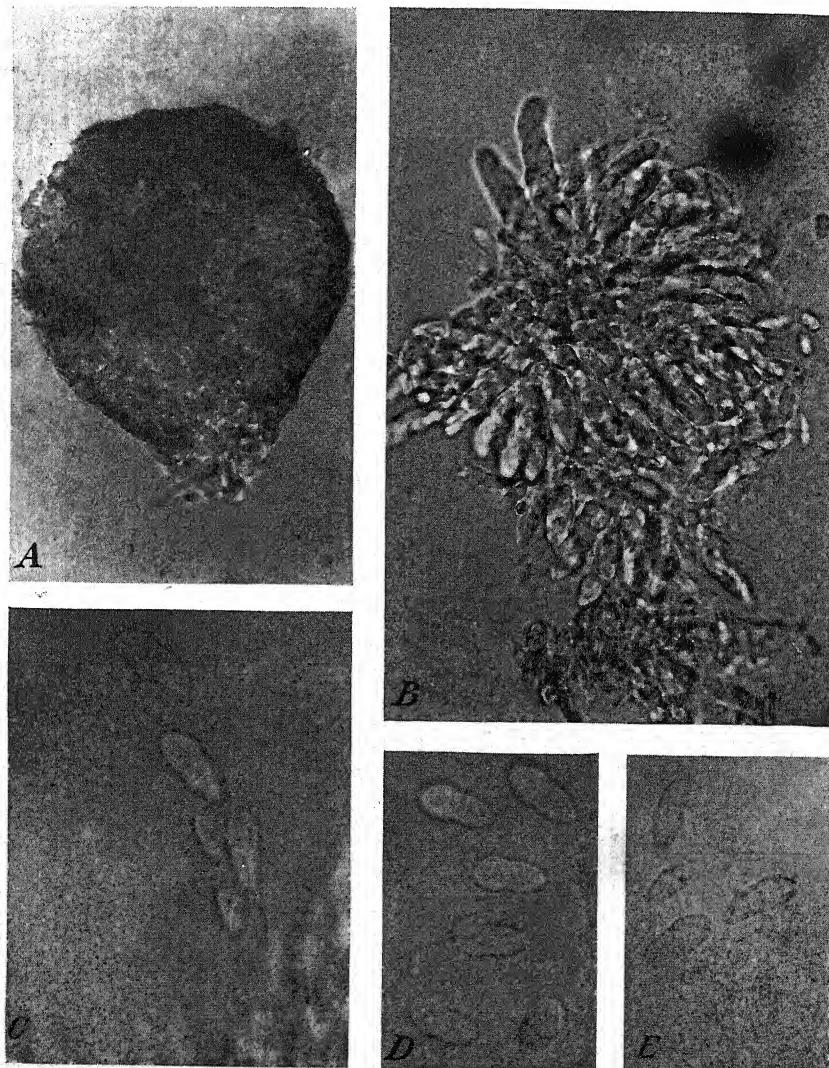
FIGURE 5.—*Mycosphaerella citrullina:* A, Single perithecium, ×430; B, contents
of single perithecium, showing asci in compact mass, ×430; C, ascospores
leaving ascus, ×900; D and E, free ascospores, ×900.

of the lesions.   Occasionally, however, perithecia are the predominant
type of fruiting structure on lesions resulting from artificial inoculation.

Single mature perithecia are occasionally observed as tiny black
specks deep in the mesophyll, but they are more frequently found in
the outer rind tissues only a little way beneath the epidermis (fig. 4, D).

On older areas of the lesion they develop in large numbers and are nearly free or have only their bases embedded (fig. 4, *E*).

The perithecia are nearly spherical to slightly elongated, with single papillate ostioles and rather heavy, nearly black walls (figs. 4, *D* and *E*, and 5, *A*). They vary greatly in size. The average diameter of those that developed on cucumber fruit lesions resulting from both natural and artificial infections was 89μ. On naturally infected chayote fruits they ranged from 91μ to 158μ with an average diameter of 124μ. On Hubbard squash lesions, the perithecia had an average diameter of 161μ and a range of 108μ to 224μ. However, isolates obtained from squash lesions, when inoculated into cucumber fruits, produced perithecia that ranged from 71μ to 110μ in diameter and averaged 87μ.

The asci tend to remain joined together at the base (fig. 5, *B*) and can best be demonstrated by partly crushing the perithecium. They are clavate-cylindrical to cylindrical and are without paraphyses. Those from cucumber lesions were found to average 10.0μ by 52.3μ with a usual range of 8.9μ to 10.6μ by 44.3μ to 76.1μ.

The asci (fig. 5, *B* and *C*) contain a single series of eight two-celled hyaline spores. The ascospores (fig. 5, *C–E*) are oblong-fusoid or fusoid, prominently constricted at the septum, with the two cells unequal. They average 6.0μ by 12.9μ, with a usual range of 4.6μ to 8.0μ by 9.2μ to 15.9μ.

DEVELOPMENT IN CULTURE

The pathogen was isolated from cucurbit fruits in Petri-dish cultures by means of tissue plantings from the advancing edge of lesions and by spore dilutions from exuded conidia. Transfers were then made from the margin of plate colonies to test-tube slants. The culture medium used was 1.5-percent potato-dextrose agar.

Although some of the isolates thus obtained eventually sporulated abundantly in culture, none did so at once, and many isolates that produced fruiting bodies only sparingly constantly remained nearly sterile. Grown on plates of potato-dextrose agar held continuously at 70° to 75° F. (fig. 6, *A*), the aerial mycelium of the poorly sporulating isolates is at first white and then gradually darkens through shades of gray to nearly black. It may range from relatively sparse to very abundant, developing uniformly or frequently with irregularly scattered cottony tufts and infrequently with slight concentric zones or faint radial lines.

Within the culture medium the mycelium is greenish black in mass and develops rapidly so that the center of the culture is prominently darkened before the plate is entirely covered by the surface mycelium. Through continued development of the substrate mycelium, the darkened area gradually extends outward to the limits of the plate, and a thin, slightly firm, felty, black fungal mat is formed at the surface of the culture medium over the entire plate. In many instances the aerial mycelium over part of the culture is appressed to the moist surface and becomes part of the mat. Frequently the fungal mat is raised and thickened at the central area of the plate. Darkening of the culture does not always proceed uniformly from the center outward but may appear in irregularly scattered areas. In all instances, however, the entire plate eventually becomes completely covered with the black fungal mat. Such fruiting bodies as may develop are found

scattered irregularly and inconspicuously over the plate and occur either singly or in tiny clusters.

Attempts were made to induce ready sporulation of these isolates by growing them on corn-meal and oatmeal agar plates and tubes, on
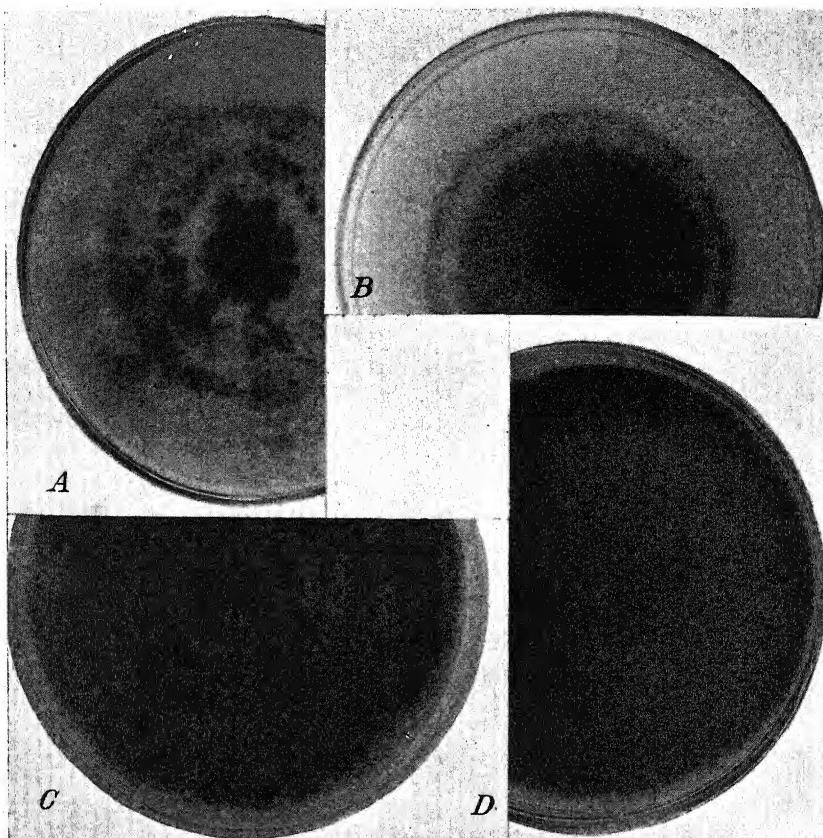
FIGURE 6.—Petri-dish cultures of *Mycosphaerella citrullina* on potato-dextrose agar. *A*, Poorly sporulating isolate from domestic Hubbard squash; aerial mycelium will eventually turn nearly black; culture 8 days old. *B*, Abundantly sporulating isolate from Puerto Rican cucumber, with sparse mycelium extending nearly to periphery of culture dish; profuse pycnidial development over entire culture, with perithecia (not distinguishable in illustration) beginning to develop near center of plate; darkening of culture has occurred in central one-half to two-thirds of plate; culture 8 days old. *C*, Abundantly sporulating isolate originally developed by sectoring from squash isolate shown in *A*; culture 14 days old, with numerous pycnidia and perithecia throughout the culture; creamy masses of exuded conidia are particularly abundant in central area. *D*, Same isolate as shown in *B*; culture 14 days old, with pycnidia and perithecia developed in abundance throughout the culture; pink masses of exuded conidia conspicuous over much of culture.

sterilized green-bean sections in tubes, sterilized cucumber rind sections in tubes and flasks, and sterilized stems of cantaloup, cucumber, squash, and watermelon, respectively, in tubes. Other tests, conducted with potato-dextrose agar as the culture medium, included cutting up plate cultures into small pieces, growing plate and tube cultures in

the dark for long periods, and exposing plate cultures to a wide range of temperatures during the course of other studies. In none of these attempts was ready sporulation induced in culture, although a few fruiting bodies similar to those produced from time to time by some of the isolates on potato-dextrose agar were occasionally observed in scattered areas of corn-meal agar and oatmeal agar plates and tubes.

During the early part of the work, those few isolates that first sporulated abundantly in culture appeared to do so more or less by chance. At that time 100 to 200 isolates were carried in culture on potato-dextrose agar plates and tubes for varying periods of time. After remaining almost completely sterile for some time, 2 of the isolates suddenly developed abundantly sporulating sectors in new plate cultures. Transfers from these sectors yielded isolates that have remained freely sporulating through many successive transfers. These, together with 20 other isolates, were then carried along as permanent stock cultures. Nearly a year and a half later 2 other isolates of the group of 22 likewise gave rise to the abundantly sporulating stage of the pathogen. Both were growing on potato-dextrose agar plates at the time. One developed a profusely sporulating sector in a plate held at 70° to 75° F. The other became moderately sporulating in the later growth that was made at 70° to 75° in plates previously held for 2 weeks at 45°. By repeated transfer from areas of the plate cultures where fruiting bodies were most abundant, the isolate eventually became profusely sporulating.

During the latter part of the work, a series of isolations was made from fruit lesions on Puerto Rican cucumbers in order to determine more definitely what proportion of the isolates would sporulate freely in culture. These were kept under observation for several weeks after transfer to tubes of potato-dextrose agar. In no case was there any evidence of abundant sporulation. After this it became necessary to set the cultures aside for 7 to 8 months; by the end of that period they had become nearly dried out. Upon resumption of the work the entire contents of the tubes were removed with a scalpel to a sterile Petri dish, and either a small piece or the entire mass was transferred to a plate of potato-dextrose agar that was held at 70° to 75° F. Within 2 to 3 weeks after transfer, 44 of the 108 isolates so tested had developed moderately to abundantly sporulating areas or sectors in the plate cultures. During subsequent transfer the abundantly sporulating stage of the pathogen was established in culture for a number of these 44 isolates. Others remained moderately sporulating, while a few never produced fruiting bodies more than sparingly.

These tests suggest that drying out of cultures favors the development of the abundantly sporulating stage of the pathogen. Observations made during the course of the work likewise indicate that once an isolate has begun to produce fruiting bodies more than sparingly it is frequently possible to bring the isolate into the completely sporulating condition by continually transferring from areas of the plate where the fruiting bodies are most abundant.

Although ordinarily there is no difficulty in maintaining an isolate in the abundantly sporulating condition once it has attained that stage, one such isolate did develop a sterile sector in a plate culture on potato-dextrose agar. Transfers from this sector have remained sterile.

On plates of potato-dextrose agar those isolates that have become abundantly sporulating in culture (fig. 6, *B–D*) produce a thin, light-colored mycelium that grows appressed to the agar. Practically no aerial mycelium develops. Pycnidia are formed early (fig. 6, *B*) and in great abundance near the surface. They are at first of light color, only slightly darker than the mycelium. Spores exuded from them pile up on the surface of the agar, giving the cultures a granular appearance. The color of the culture at this time is "light ochraceous-salmon." Before the fungus has grown to the periphery of the plate the central part of the culture becomes greenish black because of the darkening of the mycelium and pycnidia.

Perithecia, dark and somewhat smaller than the pycnidia, are slower to develop and are found first in the older, central portion of the plate. The cultures eventually become completely black (fig. 6, *C* and *D*), with inconspicuous radial or concentric patterns. Both pycnidia and perithecia (the former in greater abundance) are at this time found crowded in large numbers over the entire plate and together they constitute most of the fungus growth. Slightly pinkish masses of exuded conidia are scattered over the surface of the culture and under moist conditions may run together to form sticky or creamy spore deposits of irregular pattern. The pycnidia are for the most part similar in shape and structure to those produced in lesions on cucurbit fruits although they are somewhat smaller, falling mostly within a range of 80μ to 120μ in diameter. Larger pycnidia with two to four mouths are commonly found in smaller numbers interspersed among these. The conidia are mostly nonseptate, although a small percentage are uniseptate. In shape the nonseptate conidia are similar to but more variable than those produced on cucurbit fruits. They are also of smaller size, with a usual range of 2.1μ to 4.2μ by 5.0μ to 7.6μ, averaging 3.2μ by 5.7μ. The uniseptate conidia average 4.3μ by 9.5μ, with a usual range of 3.8μ to 5.0μ by 8.4μ to 10.9μ, and are therefore slightly smaller than those from fruit lesions.

The perithecia averaged 83μ in diameter and, although slightly smaller, were similar in shape and structure to those produced on cucurbit fruits. Both asci and ascospores were identical in size and shape with those found on fruit lesions.

In plate cultures where fruiting bodies are not produced abundantly, perithecia are frequently found in compact, somewhat spherical clusters 250μ to 450μ in diameter, with individual perithecia not clearly differentiated. It is not unusual to find asci of much greater length than normal in these clusters. Compact masses of pycnidia as large as 450μ to 850μ in diameter are likewise frequently observed in cultures that are only moderately sporulating.

<div align="center">IDENTITY</div>

Chester (*8*) observed leaf spots and a vine blight of watermelon and described the fungus associated with these symptoms as *Phyllosticta citrullina* n. sp. After noting that some of the spores were uniseptate, he stated that it was questionable whether the fungus might not be classified as an *Ascochyta*. In a later paper (*9*) he reported finding perithecia of a *Sphaerella* associated with *Phyllosticta* pycnidia that developed on watermelon leaves in a moist chamber.

Only a meager description of the *Sphaerella* stage was given, and the species was not determined.

Smith (*32*) made studies of a cucurbit disease that he observed on leaves and fruit of squash and pumpkin and leaves of cucumber and cantaloup. He considered the pathogen identical with Chester's fungus, which he renamed *Ascochyta citrullina* (Chester) C. O. Sm. Smith likewise reported finding perithecia of a *Sphaerella* on stems of squash and presented considerable evidence to show that it was the perfect stage of *A. citrullina*. Although only briefly characterizing the fungus, he named it *Sphaerella citrullina* (Chester) C. O. Sm.

Grossenbacher (*12*) reported a vine wilt of greenhouse muskmelon. After comparing the pathogen with herbarium material from the Delaware Agricultural Experiment Station, he concluded that it was identical with Smith's *Sphaerella citrullina*, which he then described more completely and renamed *Mycosphaerella citrullina* (C. O. Sm.) Gross. He stated that the *Sphaerella* reported by Chester may have been the same fungus.

Grossenbacher expressed doubt concerning the relation of Chester's *Phyllosticta citrullina* to *Mycosphaerella citrullina*. He therefore redescribed the imperfect form of the latter; and, since he did not find it on the leaves and therefore not in spots, he transferred it from *Ascochyta* and renamed it *Diplodina citrullina* (C. O. Sm.) Gross.

The imperfect form of *Mycosphaerella citrullina* when producing only nonseptate conidia might readily be mistaken for a *Phyllosticta*. This has been pointed out by Chupp (*10*), who stated that there is no assurance that a distinct species of *Phyllosticta* occurs on cucurbits although several species have been described on members of this family. Longrée's observations [8] tend to support this conclusion.

In reporting a vine canker of hothouse cucumbers, Massee (*17*) identified the pathogen as *Mycosphaerella citrullina* although he had observed only the imperfect form of the fungus. On the basis of limited cross-inoculation tests he concluded that the cucumber pathogen was also responsible for a similar stem canker of greenhouse tomatoes. After the appearance of Massee's paper, a number of other British reports were made of the tomato disease under the name "melon or cucumber canker" (*19, 20*) and "cucumber and tomato canker" (*1, 2, 3, 11*). Brooks and Price (*6*), in England, and Schoevers (*29*), in Holland, made studies of the tomato disease and likewise referred the pathogen to *M. citrullina*. However, work of Klebahn (*16*) and of Brooks and Searle (*7*) showed conclusively that the tomato pathogen was identical with *Diplodina lycopersici* Hollós and was therefore distinct from the cucurbit fungus. Klebahn observed and studied the perfect stage of the tomato pathogen, which he described as *Didymella lycopersici* (Hollós) Kleb. (*16*).

Most investigators have accepted Grossenbacher's usage (*12*) although, according to Keissler (*15*), *Ascochyta citrullina* (Chester) C. O. Sm. is identical with *A. cucumis* Faut. and Roum., which was described from leaves of cucumber (*28*) during the same year that Chester (*8*) described *Phyllosticta citrullina*. Keissler likewise considered *A. melonis* Potebnia (*23*), which was found attacking all above-ground parts of muskmelon, to be identical with *A. cucumis* Faut. and Roum. Potebnia, however, found his *Ascochyta* associated

[8] LONGRÉE, K. MYCOSPHAERELLA CITRULLINA ON ORNAMENTAL GOURDS (CUCURBITA PEPO). U. S. Bur. Plant Indus., Plant Dis. Rptr. 24: 222–224. 1940. [Processed.]

with a perfect form possessing paraphyses. This he identified as *Didymella melonis* Pass. and apparently assumed that it represented the perfect stage of *A. melonis*.

During the present studies it was found that the cucumber pathogen was identical with that isolated from domestic squash affected by black rot. Although some variation in the size of spores and fruiting bodies was noted between the measurements for both the cucumber and the squash pathogen and those reported for *Mycosphaerella citrullina* by Grossenbacher (*12*), Hemmi (*13*), and Longrée,[9] the differences were not great. The fungus that was found throughout the course of these studies to be responsible for the decay of West Indian cucurbit fruits is therefore considered to be *M. citrullina.*

TEMPERATURE RELATIONS

Occasionally in reports on the prevalence of gummy stem blight of cucumbers and melons the statement is made that the disease is favored by high temperatures. Kauffman (*14*) found that the pathogen from squash made only slow growth on corn-meal agar when held mostly at temperatures of 4° to 8° C., with an occasional upward range to 10°. These appear to be the only references in the literature to the relation of temperature to the growth of the pathogen or the development of the disease.

A study was therefore made on the effect of temperature on the growth of *Mycosphaerella citrullina* in Petri dishes containing potato-dextrose agar. In each test five cultures were used at each temperature. The diameters of the colonies were measured after 4 days at temperatures ranging by 5° intervals from 35° to 95° F; after 1 week at 35° to 60°; and after 2 weeks at 35° to 50°. Ten such tests were conducted; in each a different isolate was employed. These included seven obtained from Puerto Rican and Cuban cucumbers, two from Puerto Rican chayote, and one from domestic Hubbard squash.

The average results of the 10 tests are shown in figure 7, *A* to *C*. At the end of 4 days no growth had occurred at either 35° or 40° F. Slight growth had taken place at 45° and an increasing rate of growth was noted with increase of temperature from 45° to 80°. Maximum growth occurred at 80°. Growth fell off rapidly from 80° to 90°, with little growth at the latter temperature. No growth occurred at 95°.

No growth was found at 35° F. after 1 week. At 40° there was slight growth and at 45° slight to moderate growth. The rate of growth increased rapidly with increase of temperature from 50° to 60°. At the end of 2 weeks no growth had occurred at 35°, slight growth had taken place at 40°, moderate growth at 45°, and extensive growth at 50°. Cultures held at 95° showed no growth in 2 weeks; cultures at 35° showed no growth after 6 weeks.

The effect of temperature on the development of decay was determined by holding inoculated cucumbers at a series of temperatures ranging by 5° intervals from 35° to 65° F. The cucumber fruits were disinfected by immersing them for 30 minutes in a 1 : 1,000 solution of mercuric chloride; they were then rinsed in sterile water and placed in sterilized moist chambers. The inoculum consisted of small blocks

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[9] See footnote 8, p. 207.

of 2-week-old potato-dextrose agar plate cultures of the cucumber pathogen. Wound inoculations were made at two points on each of the eight cucumbers used at each temperature of a given test. Half of the cucumbers were removed at the end of 1 week and half at the end of 2 weeks. Measurements of the diameters of the decay lesions were made after cutting the cucumbers transversely at the point of inoculation. The results obtained by averaging six such tests are shown in figure 7, *D* and *E*.

At the end of 1 week there was no development of decay at either 35° or 40° F. Decay was slight at 45° and moderate at 50°. From
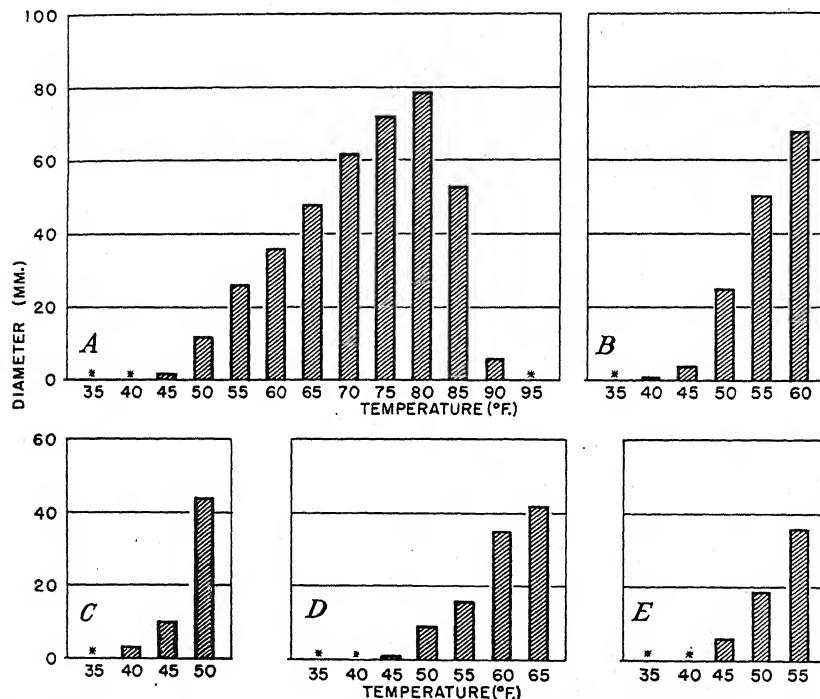
FIGURE 7.—*A–C*, Temperature relations of *Mycosphaerella citrullina* as determined by average diameter of 50 colonies on potato-dextrose agar after (*A*) 4 days, (*B*) 1 week, and (*C*) 2 weeks; *D* and *E*, temperature relations of mycosphaerella black rot as determined by average diameter of 48 lesions on cucumber fruits 1 week (*D*) and 2 weeks (*E*) after inoculation. Asterisk (*) indicates that there was no growth for the particular period.

50° to 65° the rate of decay increased rapidly with increase of temperature, so that by the end of 1 week the lesions were 42 mm. in diameter at 65°.

Two weeks after inoculation decay had not developed at 35° or 40° F., although moderate-sized lesions were evident at 45°. Rapid increase in decay occurred at 50° and 55°, so that lesions at 55° were 36 mm. in diameter.

In other tests advanced lesions placed at low temperatures showed no further enlargement at 35° F. after 2 weeks and very slight enlargement after 1 week at 40°.

CONTROL

Observations made during the present study suggest that much of the mycosphaerella black rot found in Puerto Rican and Cuban cucumbers on the market had its origin in field infections. Control of the disease in the field is essential in any program for its control on the market. Seed treatments, because the pathogen is seed-borne, and the application of bordeaux mixture spray or copper-lime dust are the chief field control measures recommended for cucumbers *(37)* and other cucurbits *(34)*.

During the course of the market studies it was found that cucumber fruits could readily be inoculated with *Mycosphaerella citrullina* at skin breaks and wounds. Although the tests were somewhat limited, in no case was there any evidence of infection having occurred through the unbroken skin of mature fruits. Early tests by Meier[10] indicated that infection could be accomplished by puncturing sound cucumbers with one's fingernail while handling cucumbers on which the fungus was fruiting. Therefore, care should be taken to avoid rough handling during harvesting and packing.

No experiments have been conducted to determine how much infection occurs during the picking and packing operations. However, the rapid and abundant production of spores on black rot lesions suggests that many cucumbers harvested from diseased fields might well be expected to carry a heavy spore load.

Distribution of spores over the surface of cucumbers could readily occur during washing operations, particularly if diseased cucumbers are overlooked and thus reach the wash water. The possibility of control through adding fungicides to the wash water or through the use of a fungicidal bath should be studied.

A number of investigators have pointed out that in the field the disease is usually more serious and spreads more rapidly when moisture is high. Cucumbers that are harvested during rainy periods or shortly thereafter are therefore more subject to decay during the marketing period. Regardless of conditions prevailing in the field, cucumbers and other curcurbits should be moved to market as soon as possible after being harvested.

One of the most important measures for reducing market losses from mycosphaerella black rot in cucumbers shipped from Puerto Rico and Cuba is to precool them to 50° F. or below. The rapidity with which the decay develops between 55°–85° emphasizes the importance of prompt cooling. The results of the temperature studies show definitely that the decay can be almost completely held in check at 45°, although a carrying temperature of 40° would be preferable. In this connection it should be remembered that prolonged exposure of cucumbers to temperatures of 45° or lower may cause low-temperature break-down *(24, 27)*. However, for short transit periods of 1 week or less there is probably little danger of low-temperature injury at 40°–45°, the temperatures here recommended for Puerto Rican and Cuban cucumbers.

SUMMARY

During investigations extending from 1938 to 1942, inclusive, mycosphaerella black rot, caused by the fungus *Mycosphaerella ci-*

trullina (*C. O. Sm.*) Gross., was found to be chiefly responsible for the serious losses from decay frequently observed in Puerto Rican and Cuban cucumbers arriving on the New York, N. Y., market.

A review of the literature shows that the pathogen is widely distributed and attacks many cucurbits of economic importance. Symptoms as reported may include spotting of the leaves; production of cankers on the stems, petioles, and fruitstalks; decay of the stem; wilt of the vine; and rotting of the fruit.

Although market losses were greatest on Puerto Rican and Cuban cucumbers, the decay was also found affecting balsam pear, chayote, Chinese preserving melon, Yellow Crookneck squash, and watermelon from Cuba; chayote from Jamaica and Puerto Rico, and muskmelon presumably from Venezuela. During extensive observations on the New York market the decay was noted only once on domestic cucumbers (in a carlot from Georgia), and in no instance was it found on any type of domestic muskmelon. The decay came to attention twice on domestic Hubbard squash (from Virginia and from Massachusetts) but it was not observed on domestic watermelon during the course of limited observations.

Symptoms of mycosphaerella black rot on cucumbers are described and illustrated in detail and, to a lesser extent, symptoms on balsam pear, chayote, dishcloth gourd, muskmelon, and squash.

An illustrated account is given of the development of the pathogen on cucurbit fruits and its cultural behavior on natural and artificial media.

Temperature-relation studies showed that the pathogen makes most rapid growth in culture at 65° to 85° F., with the maximum at 80°. No growth occurred at 95° even in 2 weeks. Growth was markedly reduced at 45°, while only slight growth took place in 1 to 2 weeks at 40°. No growth occurred at 35° in 6 weeks.

The development of decay on cucumber fruits was very slow at 45° F. No decay took place at 35° or 40°. Rapid increase in decay occurred at 50° and 55°.

Measures suggested for reducing market losses (particularly for Puerto Rican and Cuban cucumbers) include field control through seed treatment and spraying, care in picking and packing to avoid mechanical injuries, prompt handling after picking, precooling to 50° F. or somewhat lower, and maintaining temperatures of 40° to 45° during the period of transit to the market.

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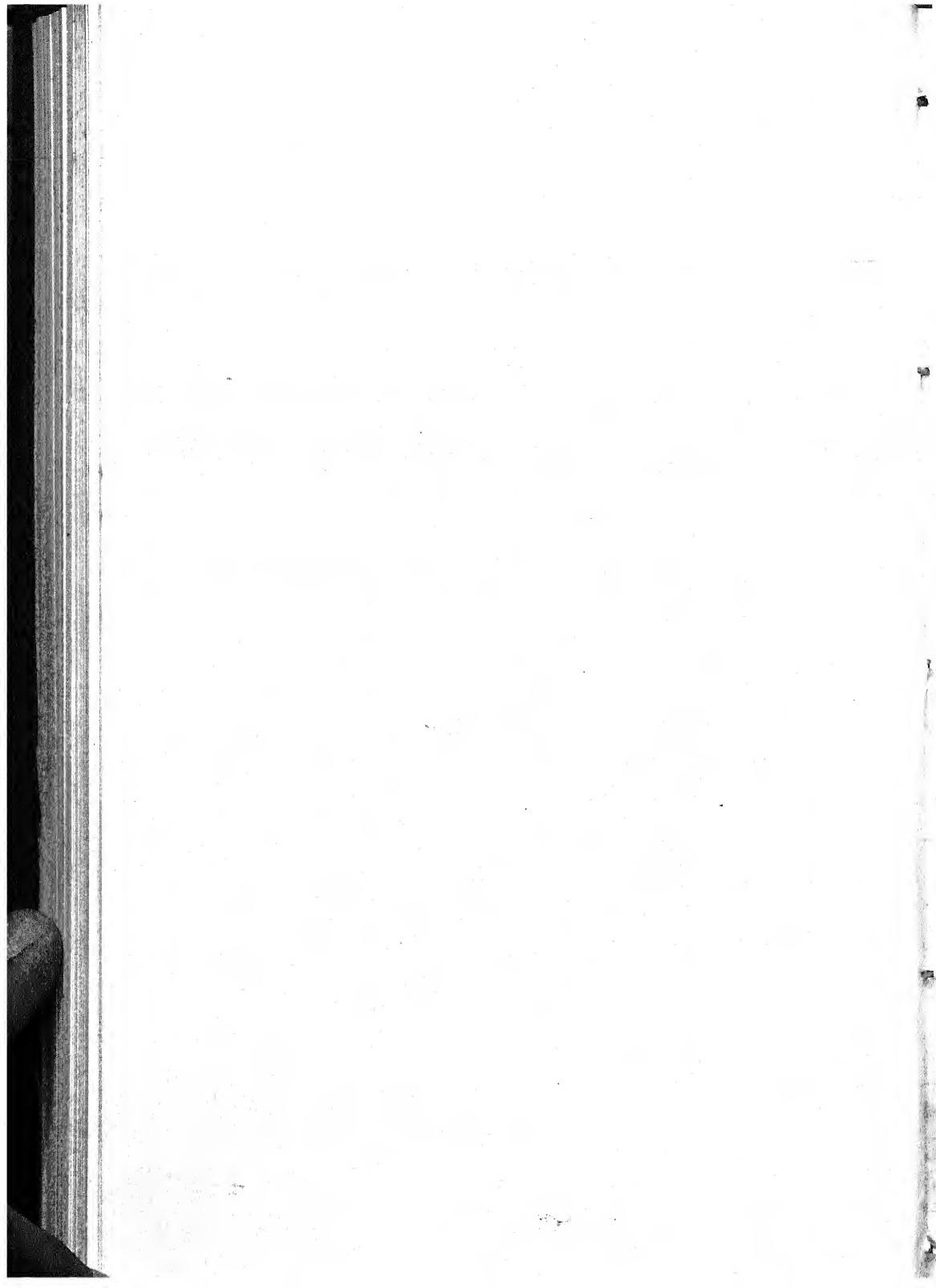
EMBRYOLOGY OF THE TUNG TREE [1]

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INTRODUCTION

The initiation of the pistillate flower, the development of the ovary, the growth of the fruit and its parts, and the general aspects of the morphology and anatomy of the fruit of the tung tree (*Aleurites fordi* Hemsl.) have been reported in earlier papers (*1, 3, 5, 6*).[2] However, cytological details of megasporogenesis, embryo sac formation, and embryo growth have not been described, and it is the purpose of this paper to supplement the earlier papers with this information.

REVIEW OF LITERATURE

Botanical information pertaining to the genus *Aleurites* is limited to a few publications concerned with elementary descriptive morphology. To date there has been no attempt to study the cytology of the genus. However, considerable data have been published on species of various other genera in the family Euphorbiaceae. Modilewski (*7, 8, 9*) has described embryo sac development in several species of *Euphorbia*. He reported that the ovule of *E. procera* has a projected nucellus, which is in direct contact with papillate cells of a specialized obturator. He further stated that the ovules of most Euphorbiaceae contain a single megaspore cell that divides to form four daughter cells, of which the chalazal cell develops a normal eight-nucleate embryo sac. Lyon (*4*) likewise reported a projected nucellus in *E. corollata*, and Schlotterbeck (*12*) described a similar structure in *Croton tiglium*. These structures are to be compared with the similar structure found in tung.

According to a summary of types of embryo sac development in the Euphorbiaceae by Schnarf (*13, pp. 220–221*), the majority of the species of that family have a normal eight-nucleate embryo sac development.

D'Amato (*2*) reported that in *Euphorbia paralias, E. falcata* var. *acuminata, E. pubescens, E. pithyusa* var. *ovalifolia, E. characias,* and *E. amygdaloides* the archesporium is usually unicellular, that the female gametophyte is produced by a chalazal megaspore, and that

most species show the *Scilla* type of gametophyte either regularly or occasionally. The fertilized egg usually rests, while the triple fusion nucleus immediately divides to give rise to the endosperm. Antipodals degenerate early.

A study of the pistillate flower and developing fruit of tung by McCann (*5*) included the general aspects of the morphology of the ovary and its parts and the growth of these parts into the mature fruit and seed. The gross and the vascular anatomy of the fruit and seed were discussed. The development of the pistillate flower begins 5 to 10 months before anthesis. The primordia may be initiated at any time between late May and late October. All pistillate buds develop to the characteristic cup stage, consisting mainly of carpellary tissue, before the winter months. There is no development of the placenta and septa until the renewal of growth in the spring, usually about the first part of February. The first indication of resumed growth in the pistil is observed in the edges of the carpellary cup; about the same time the axial placentae begin to develop as mounds of tissue rising in the base of the cup. Locular partitions, or septa, rise between the incipient locules and connect the ovary wall to the placentae. The placentae, septa, and ovary wall eventually converge at the apex and close over the locules. The ovary at anthesis is superior and polycarpellary, with axial placentae.

METHODS AND MATERIALS

From September 1938 to June 1942 terminal buds, flowers, and fruits were collected from tung trees within a 25-mile radius of Bogalusa, La., and were fixed in a variety of fixatives. Beginning January 1, 1939, weekly collections were made from each of thirty 5- to 12-year-old seedling trees selected for their variation in habitat and growth characteristics. At least 3 buds were taken from each tree at weekly intervals through December 1939. In addition, during each collection period 30 to 50 buds were collected at random from trees in the immediate vicinity of the selected seedlings.

During 1940 monthly collections were made from miscellaneous trees except from May to October, when collections were made at weekly intervals. These collections were studied by means of a dissection technique (*6*).

During the flowering periods of 1939–41 hand-pollinations were made and after pollination fruits were collected at approximately 12-hour intervals for 1 week, then at 24-hour intervals for 2 weeks, and at weekly intervals for 2½ months.

Pistillate flowers were dissected from the terminal buds collected during the dormant period up to anthesis, dehydrated in a series of ethyl and butyl alcohols, embedded in paraffin, and sectioned on a rotary microtome at 8μ to 15μ. Individual ovules were dissected from fruits collected after anthesis. These were treated in the same manner as the pistillate buds. Some whole fruits collected 6 weeks after pollination were sectioned either as a whole or in part.

Sections were stained in safranine 0 and fast green, crystal violet, iron-alum haematoxylin, and crystal violet and eosin.

Photomicrographs were made with 16-, 32-, 48-, and 72-mm. Micro Tessar lenses and with 2-, 4-, and 8-mm. apochromatic objectives in

combination with $10 \times$ or $15 \times$ compensating oculars. A bellows extension was used in making all photographs.

Drawings were made with the aid of a camera lucida or by direct tracing of enlarged photographs.

OBSERVATIONAL DATA

INITIATION OF OVULE

Ovule primordia usually make their appearance in late February, at the time or slightly before the locules are closed over by growth of the carpel walls. The ovule primordia appear on the axial placenta near the apical end of each locule and consist of two integuments surrounding a large nucellar tissue. The primordia develop in the manner typical of the normal anatropous ovule except that, in the very early stages of primordia initiation, the nucellus protrudes through and beyond the micropylar opening formed by the integuments. The protruding nucellus is also typical of other Euphorbiaceae (*10, pp. 184–189*).

MEGASPOROGENESIS

About 2 weeks before anthesis the megaspore mother cell is differentiated from a hypodermal cell of the nucellus (fig. 1, *A, B*). At this time it is located near the apex and is readily distinguishable from other cells of the nucellus by its enlarged nucleus, elongated appearance, and rather extensive vacuolization. As the megaspore elongates in preparation for division, it becomes more deeply imbedded, so that, by the time the nucleus divides, the cell lies at the approximate center of the ovule (fig. 1, *C, D*). Two transverse divisions take place, giving rise to a row of four megaspores, the outer three of which degenerate (fig. 2, *A–D*). The chalazal megaspore enlarges and elongates in preparation for the third division during which two nuclei form; one of these orients itself in the micropylar end and the other in the chalazal end of the embryo sac (figs. 1, *E, F,* and 2, *D, E*). The fourth division is periclinal, giving rise to two nuclei at each pole (fig. 2, *F*). Each nucleus of the sac then goes through the fifth and final division to form eight nuclei (fig. 2, *G*).

The nuclei then orient themselves in the conventional manner of the normal eight-celled embryo sac, consisting of an egg and two synergids in the micropylar end, three antipodals in the chalazal end, and two polar nuclei at or near the center of the sac (fig. 2, *H*).

FERTILIZATION OF EGG AND GROWTH OF EMBRYO

The egg nucleus is fertilized between 24 and 36 hours after pollination. This was made evident by the presence of discharged pollen tubes in the embryo sac, enlarged and more darkly staining egg nuclei, vacuolization of the egg cell cytoplasm, and in a few cases the presence of the male nucleus within the egg cell and in contact with the egg nucleus (fig. 1, *H, I*).

Synergids degenerate early, sometimes even before fertilization. The antipodals are also short-lived and usually show signs of degeneration at the time of fertilization or soon after (figs. 1, *G,* and 2, *I*).

The fertilized egg does not immediately begin to divide but rests for 1 to 2 weeks. The earliest division of the fertilized egg was ob-
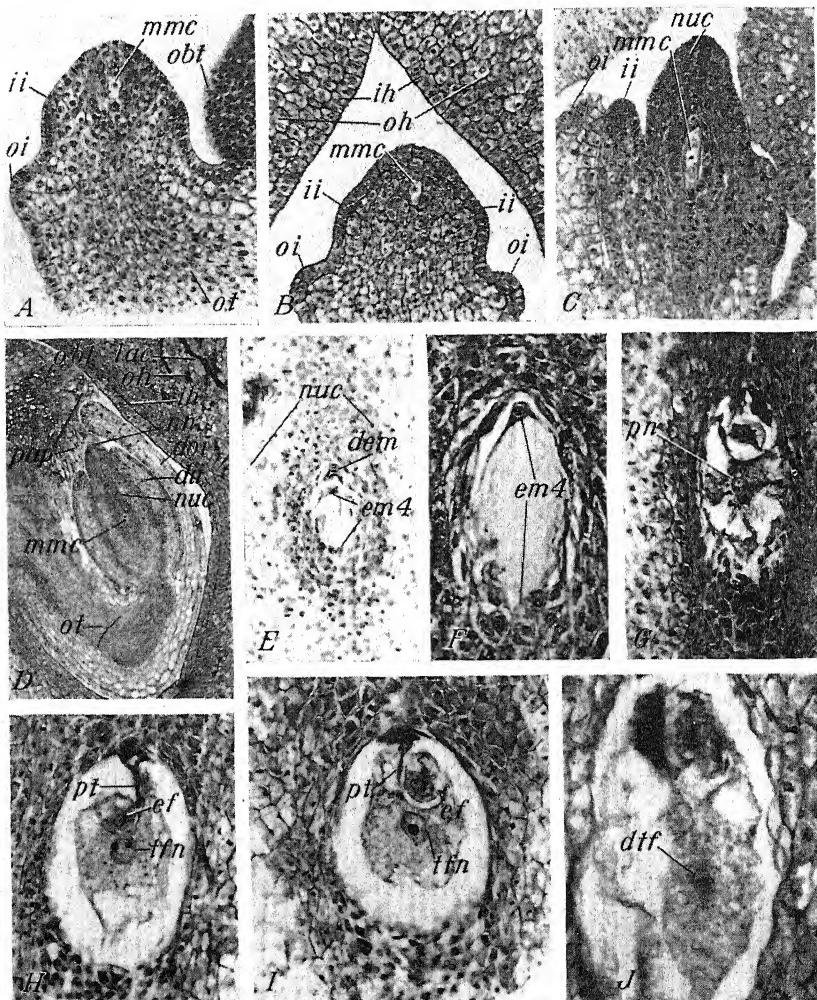
FIGURE 1.—*A*, Median radial longitudinal section of young ovule, showing megaspore mother cell and integument primordia; March 21, 1941. *B*, Median tangential longitudinal section, showing megaspore mother cell and portion of ovary wall; March 19, 1941. *C*, Longitudinal section of ovule, showing growth of integument primordia and nucellus and elongation of megaspore mother cell in preparation for first division; March 28, 1941. *D*, Median radial longitudinal section of ovule, showing integuments, nucellar neck, obturator and its papillate cells, ovular trace, and the beginning of the development of the inner hull from the inner layers of the ovary wall; April 6, 1941. *E*, Longitudinal section through ovule, showing three degenerating megaspores and two nuclei, one at each pole of embryo sac; nuclei have resulted from division of the chalaza of the four original megaspores; April 3, 1941. *F*, Four-nucleate embryo sac in which three of the nuclei are visible, two in the chalazal end and one in the micropylar end of the sac; March 28, 1941. *G*, Longitudinal section through embryo sac, showing fusion of polar nuclei in center; early degeneration of synergid cell is apparent in micropylar end of sac; April 3, 1941. *H*, Embryo sac showing presence of discharged pollen tube lying adjacent to the egg cell, 2n endosperm nucleus (possibly 3n), and two of the three degenerating antipodals; April 6, 1941. *I*, Embryo sac in approximately same stage as shown in *H*; nuclei of both egg and endosperm appear to have been fertilized by generative nuclei; April 6, 1941. *J*, Embryo sac showing first division of the 3n endosperm nucleus, 52 hours after pollination; April 8, 1941. *A*, *B*, *E*, and *G*, × 165; *C*, × 160; *D*, × 65; *F*, × 350; *H* and *I*, × 185; *J*, × 340. *dem*, Degenerating megaspores; *dii*, dorsal inner integument; *doi*, dorsal outer integument; *dtf*, first division of triple fusion nucleus; *ef*, fertilized egg; *em4*, four-celled embryo sac; *ih*, inner hull; *ii*, inner integument; *lac*, latex cells; *mmc*, megaspore mother cell; *nn*, nucellar neck; *nuc*, nucellus; *obt*, obturator; *oh*, outer hull; *oi*, outer integument; *ot*, ovular trace; *pn*, polar nucleus fusing; *pap*, papillate cells of obturator; *pt*, pollen tube; *tfn*, triple fusion nucleus.
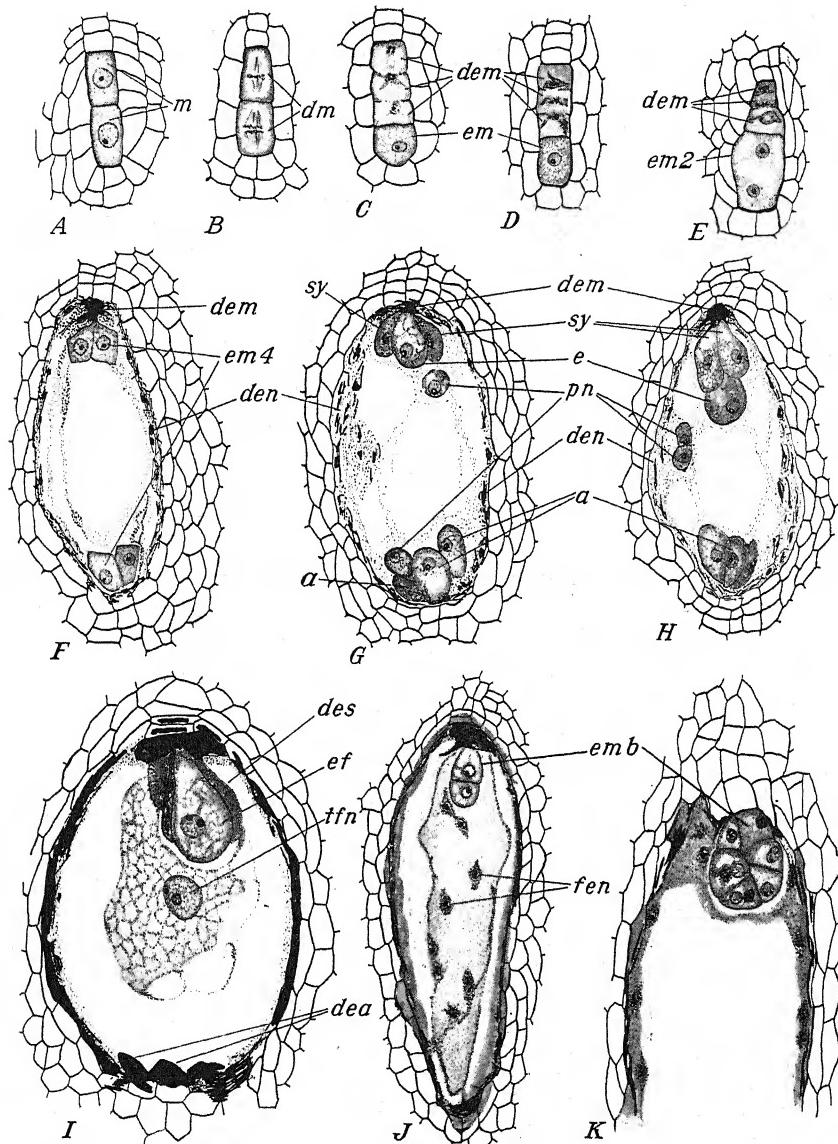
FIGURE 2.—*A*, Two macrospores resulting from the first division of the macrospore mother cell; March 28, 1942. *B*, Two macrospores undergoing the second division in the formation of the embryo sac; March 30, 1941. *C*, Row of four macrospores, the upper three showing some degeneration of cellular contents; April 2, 1941. *D*, Further degeneration of upper three macrospores and enlargement of chalazal macrospore in preparation for the third division in the formation of the embryo sac; April 3, 1941. *E*, Two nuclei resulting from the third division, and three degenerating micropylar-macrospores; April 6, 1941. *F*, Four-celled embryo sac resulting from the fourth division; April 8, 1941. *G*, Eight-celled embryo sac resulting from fifth and final division; April 9, 1941. *H*, Orientation of embryo sac nuclei in normal manner; an egg and two synergids in the micropylar end, three antipodals in the chalazal end, and two polar nuclei at or near the center of the sac; April 10, 1941. *I*, Fertilized eggs, degenerated synergids and antipodals, and triple fusion nucleus; April 27, 1941. *J*, First division of the fertilized egg surrounded by free endosperm nuclei; May 2, 1941. *K*, Eight-celled embryo surrounded by peripheral band of free endosperm nuclei; May 15, 1941. Drawings made with the aid of a camera lucida. *A* to *H*, × 390; *I* and *K*, × 170; *J*, × 150. *a*, Antipodals; *dea*, degenerating antipodals; *dem*, degenerating macrospores; *den*, degenerating nucellus; *des*, degenerating synergids; *dm*, dividing megaspores; *e*, egg; *ef*, fertilized egg; *em*, megaspore-forming embryo sac nuclei; *em2*, first two-embryo sac nuclei; *em4*, four-embryo sac nuclei; *emb*, embryo; *fen*, free endosperm nuclei; *m*, megaspores; *pn*, polar nucleus; *sy*, synergid; *tfn*, triple fusion nucleus.

served at 120 hours after pollination. The next few divisions of the embryo take place comparatively slowly. Embryos collected 2 to 4 weeks after pollination were found in the 8-cell stage; at 6, 7½, and 10 weeks after pollination, most embryos were in the 32-cell stage. Thus it appears that the embryo may rest for a month or more at the 32-cell stage. This rest period usually comes during the latter part of May or early June, depending on the season.

The first division of the fertilized egg is in a transverse plane (fig. 2, J). The subsequent divisions have not been seen, but they are such as to cause the embryo to assume a spheroid shape at the 32- to 64-cell stages.

SUSPENSOR

The suspensor of the tung embryo is multicellular (fig. 3, I). A study of the shape and position of the cells in the four- and eight-celled embryos indicates that the suspensor may develop from the upper of the first two cells resulting from the egg cell divisions. The suspensor is attached to the lower part of the nucellar neck, which extends through the micropyle (fig. 3, C, D, F–H). In the early stages of development, the connecting cells are large and thin-walled at the point of attachment. By the time the embryo begins rapid growth, usually about July 1, the suspensor has developed into a multicellular vermiform structure (fig. 3, J).

DEVELOPMENT OF ENDOSPERM

The two polar nuclei fuse just before their union with the second male nucleus, forming the $3n$ fusion nucleus. The first division of this $3n$ nucleus was observed in material collected 52 hours after pollination (fig. 1, J). At 62 hours after pollination, eight free endosperm nuclei were found. Subsequent divisions take place regularly but relatively slowly. By the first of June the endosperm nuclei, which are embedded in heavy protoplasm, form a single layer around the periphery of the embryo sac. In 1941 the first evidence of multiple layers of endosperm was observed on June 6. Numerous division figures were found in the micropylar end of the sac in the vicinity of the embryo (fig. 3, E). Cell walls began to form in the endosperm about 10 days later.

PERIOD OF RAPID DEVELOPMENT OF EMBRYO AND ENDOSPERM

About July 1 both endosperm and embryo begin to develop rapidly. Cotyledon primordia appear and rapidly extend through the embryo sac (fig. 3, I–K). The endosperm tissue develops centrifugally from the peripheral layer around the embryo sac and by late August fills the seed at the expense of the nucellus and inner integument tissue.

Since only the outer epidermis of the inner integument is concerned in the development of the shell, the role of the remaining tissue of the inner integument must be considered in relation to the developing embryo and endosperm. The nucellus occupies the center of the young ovule with the exception of the space occupied by the embryo sac. The nucellus is enclosed b yinner integument tissue. In the chalazal end of the ovule the nucellus is separated from the inner integument by the conducting tissue that extends from the ovular trace through the opening in the chalazal end of the seed coat. In the
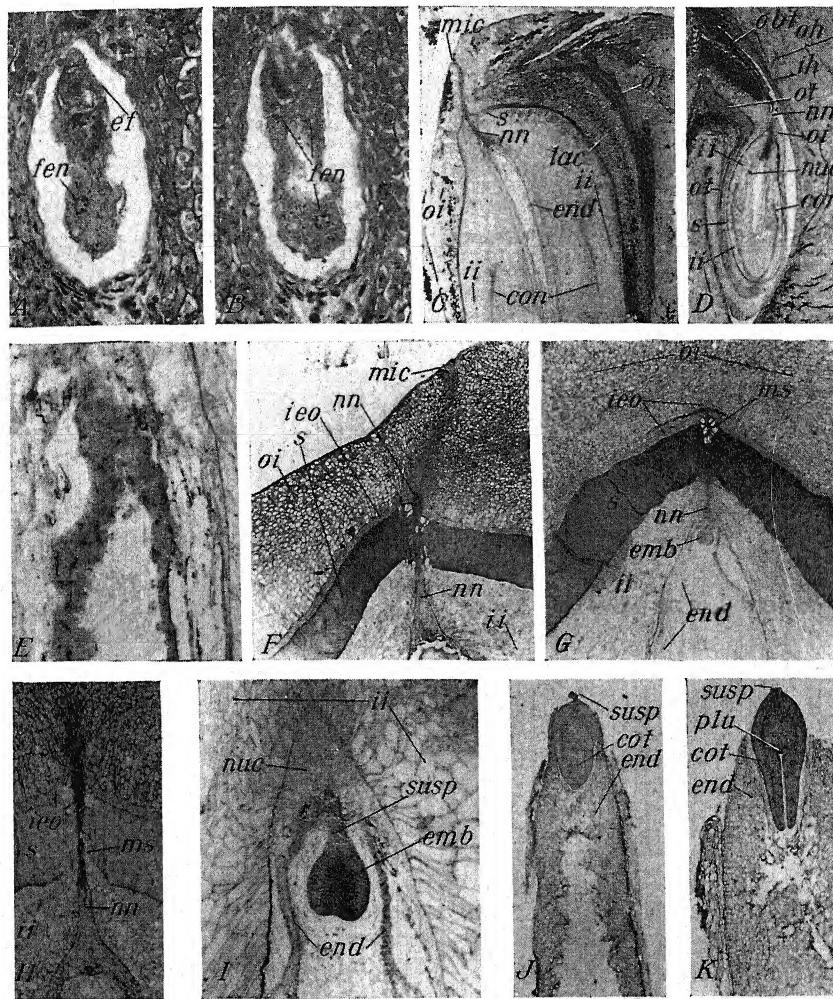
FIGURE 3.—*A, B,* Successive sections of embryo sac, showing four free endosperm nuclei and fertilized egg; April 10, 1941. *C,* Longitudinal section of micropylar half of developing ovule showing ovular trace, micropylar opening through outer integument and developing shell, extension of nucellar neck through micropylar opening, remains of nucellus lining embryo sac, and ends of conducting system extension of ovular trace embedded in inner integument tissue; May 22, 1940. *D,* Longitudinal median radial section of ovule, showing vascular supply to ovule and the connection of nucellar neck to obturator through micropyle; April 19, 1941. *E,* Longitudinal section through micropylar end of embryo sac showing simultaneous divisions of free endosperm nuclei; June 6, 1941. *F,* Longitudinal median radial section through micropylar end of seed, showing formation of shell from outer epidermis of inner integument, development of inner epidermis of outer integument as lining of the upper part of micropylar canal, and club-shaped cells of shell forming micropylar aperture through which nucellar neck extends; July 1, 1940. *G,* Longitudinal median tangential section through micropylar end of seed, showing further development of parts mentioned in *F;* note attachment of embryo to nucellar neck; June 28, 1940. *H,* Longitudinal median tangential section of micropylar opening in shell, showing extension of nucellar neck through aperture; June 21, 1940. *I,* Many-celled embryo and suspensor attached to basal position of nucellar neck; June 28, 1940. *J,* Embryo with suspensor and cotyledon primordia surrounded by endosperm cells; degenerating nucellus and inner integument cells surround the endosperm; July 18, 1940. *K.* Further development of embryo; July 16, 1940. *A* and *B,* × 180; *C* and *D,* × 10; *E,* × 80; *F* and *G,* × 15; *H, J,* and *K,* × 20; *I,* × 40.   *con,* Conducting tissue; *cot,* cotyledon; *ef,* fertilized egg; *emb,* embryo; *end,* endosperm; *fen,* free endosperm nuclei; *ieo,* inner epidermis of outer integument; *ih,* inner hull; *ii,* inner integument; *lac,* latex cells; *mic,* micropyle; *ms,* modified cells of shell at micropylar opening; *nn,* nucellar neck; *nuc,* nucellus; *obt,* obturator; *oh,* outer hull; *oi,* outer integument; *ot,* ovular trace; *plu,* plumule primordium; *s,* shell of seed, or outer epidermis of inner integument; *susp,* suspensor.

micropylar end the two tissues are separated by a more or less persistent inner epidermis of the inner integument (fig. 3, D).

As endosperm tissue develops, the nucellus, with the exception of the region making up the nucellar neck, gradually degenerates and is absorbed as the embryo sac increases in size. By the time cell wall formation begins in the endosperm, usually about the middle of June, the major part of the nucellus has been absorbed. The tissue of the inner integument, however, is more persistent, and, though the cells adjacent to its inner epidermis show evidence of collapse and distortion, the tissue is intact until the centrifugal growth of the endosperm crushes the remaining cells against the lignified shell. In the mature seed, the crushed tissue adheres to the shell as a dry, fuzzy layer and is termed the pellicle. Thus, for the most part, the pellicle is made up of remnants of inner integument but may also contain some elements of the conducting tissue and perhaps a small portion of nucellar fragments.

MICROPYLE

The micropyle is commonly defined as the orifice, or aperture, in the seed coat through which the pollen tube enters the embryo sac. Prior to and at the time of fertilization in the tung ovule the nucellus extends through the micropyle and connects with the papillate cells of the obturator (figs. 1, D; 3, D, F; 4, E). The micropylar aperture penetrates the inner and outer integuments. Later, the micropylar canal extends through the shell and the aperture formed by loosely connected modified cells (fig. 3, H). Living parenchyma tissue of the nucellar neck may be found in the micropylar canal as late as the middle of June, at which time the cells of the shell begin to lignify (fig. 3, H).

CHALAZA

The ovular trace begins to differentiate in the early stages of ovule development (fig. 1, A, D) and is present and functional throughout the development of the ovule, embryo sac, and early stages of endosperm and embryo development (fig. 3, C, D). As the outer epidermis of the inner integument differentiates into the cells of the shell, an orifice is left in the chalazal end of the shell through which the ovular trace develops (fig. 3, D). Spiral elements characteristic of vascular tissue are found in the conducting tissue extensions of the ovular trace inside the shell.

GROWTH OF SHELL

The shell of the tung seed differentiates from the outer epidermis of the inner integument. During the early development of the ovule after fertilization the shell appears to be made up of both the outer epidermis of the inner integument and the inner epidermis of the outer integument. Both layers of cells are made up of isodiametric cells with somewhat thickened cell walls (fig. 4, A). The inner layer, i. e., the outer epidermis of the inner integument, begins to differentiate into shell during the latter part of May (fig. 4, C). The cells of the micropylar region are the first to show signs of elongation. Differentiation of the remaining cells takes place rapidly. The shell attains its maximum thickness in about 1 month and the cell walls
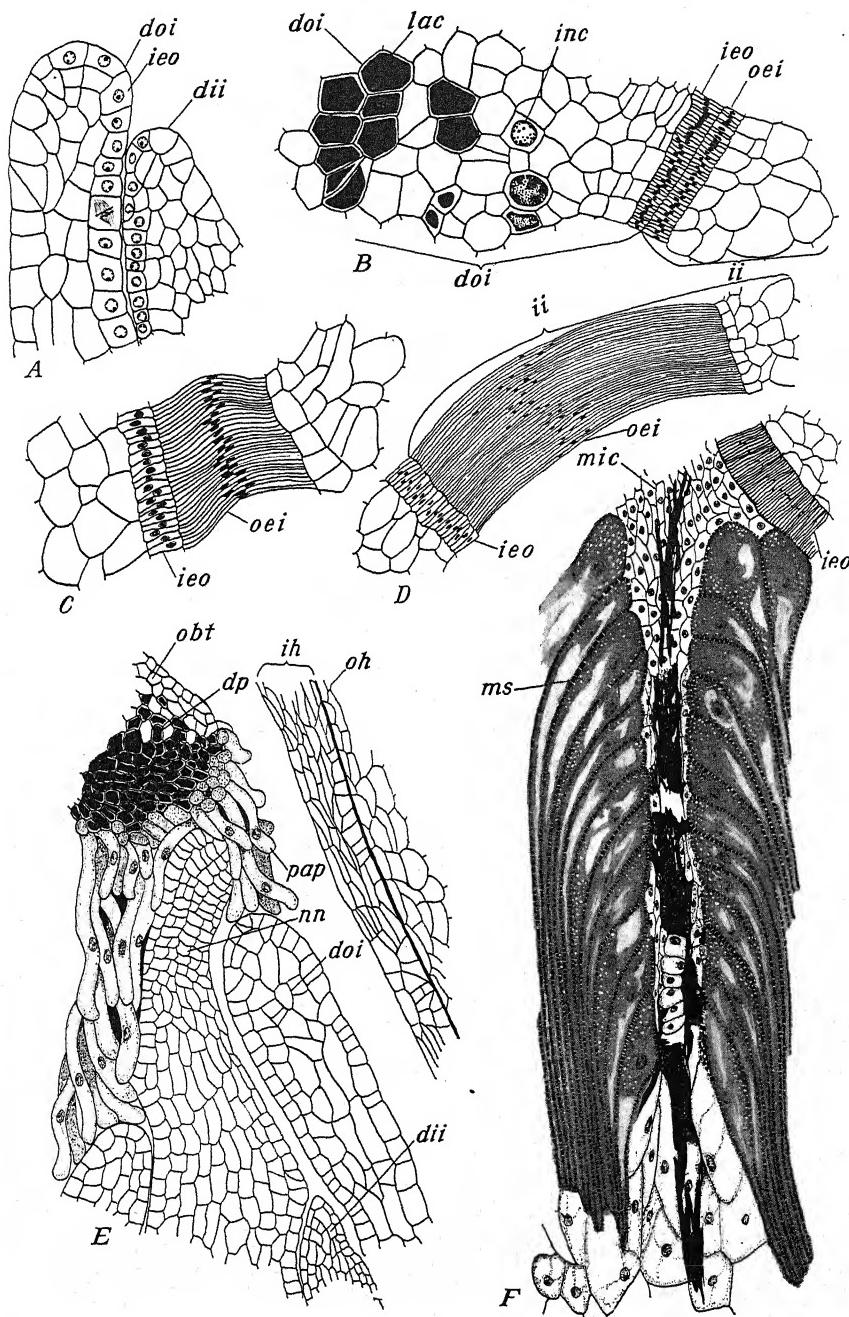
FIGURE 4.—*A–D*, Development of shell from outer epidermis of inner integument. *A*, March 28, 1941; ×385. *B*, April 25, 1941; × 170. *C*, May 22, 1941; × 170. *D*, July 15, 1941; ×90. *E*, Papillate cells of obturator in contact with nucellar neck; × 175. *F*, Micropylar opening with modified cells of shell, showing nuclei and pitted walls; × 125. Drawings made with the aid of the camera lucida. *dii*, Dorsal inner integument; *doi*, dorsal outer integument; *dp*, cells containing dense protoplasm and inclusions; *ieo*, inner epidermis of outer integument; *ih*, inner hull; *ii*, inner integument; *inc*, inclusions; *lac*, latex cells; *mic*, micropyle; *ms*, modified cells of shell at micropylar opening; *nn*, nucellar neck; *obt*, obturator; *oei*, outer epidermis of inner integument; *oh*, outer hull; *pap*, papillate cells of obturator.

immediately begin to lignify. Sections made as late as July 15 show these cell walls to be perforated with pits (fig. 4, *F*). The cells of the inner epidermis of the outer integument becomes more elongated and more closely compacted and have thicker walls than the bordering parenchyma, but they remain about one-tenth to one-fifteenth the size of the cells of the shell (fig. 4, *F*).

DISCUSSION OF OBSERVATIONAL DATA

Embryo sac formation in the tung ovule is apparently of the eight-nucleate normal type, and there are no outstanding deviations from the normal. However, there are certain accessory structures and tissues that warrant some discussion. Probably the outstanding deviation from the normal anatropous type of ovule development is the presence of a nucellar neck that extends through the micropyle. This structure is first visible at the time of the initiation of the first ovular primordium and persists until the seed is almost ripe. The neck extends beyond the ends of the integument primordia into the locular cavity and eventually comes in contact with papillate cells of the obturator immediately at the micropylar end of the anatropous ovule. Since the tissue of the obturator and its papillate extensions consist of cells having dense protoplasmic contents with great affinity for biological dyes, it is suggested that the nucellar neck and papillate cells form a bridge for the translocation of nutritive materials from the tissue of the obturator to the embryo sac. At the time of embryo sac formation and throughout the growth of the embryo, the nucellar neck is in direct contact with the suspensor and the dark-staining cells at the obturator end of the bridge are in proximity to the vascular complex in the funicular region of the placenta.

The developing ovule is also in direct contact with the main vascular system by means of an ovular trace that is recognizable from the time the first primordia of the ovule appear. At the time of fertilization the ovular trace is clearly defined as a region of elongated dark-staining cells extending the length of the anatropous ovule from the funiculus through the center of the ventral outer integument to the chalazal end. The trace turns upward abruptly at the base of the ovule and penetrates through the outer epidermis of the inner integument, where it connects with a bell-shaped system of elongated cells, which exhibit spiral elements and without doubt serve to extend the ovular trace into the ovule about three-fourths the length of the ovule. Thus the nucellus is in direct contact with the main vascular system of the axis throughout the development of the ovule and seed.

Therefore, the growing embryo may receive nourishment in any one or all of three ways: (1) It is in direct contact with a nucellar neck, which is itself rich in aleurone grains and is indirectly connected to the main vascular complex through the rich storage tissue of the obturator; (2) it may receive nourishment from the cellular contents of the degenerating nucellus encroached upon by the enlarging embryo sac; (3) it may receive nourishment from the extension of the ovular trace during and after nucellar resorption. It is also possible that the inner parenchymatous layers of the inner integuments contribute to the nutrition of the contents of the embryo sac, though it is more likely that this tissue, together with the outer integument, has some

part in the tremendous elongation of the outer epidermal cells of the inner integument, which form the shell of the seed.

There is no definite tapetum inside the shell, unless the inner epidermal cells of the inner integument immediately adjacent to the nucellar neck may be so designated. There is no thickening of the cell walls of these epidermal cells such as is usually reported for tapetal development.

The micropylar foramen lies between the tips of the outer integument and penetrates the shell by means of a small orifice formed by enlarged, pyriform, pitted cells of the shell. The foramen encloses nucellar neck tissue from the beginning of ovule formation throughout the development of the seed. Toward the latter part of seed development the parenchymatous neck tissue is crushed, so that only remnants of dark-staining material remain in the micropylar canal.

The inner epidermis of the outer integument completely surrounds the outer circumference of the shell. The cells of this layer are heavy-walled and somewhat elongated. In the region bordering the upper part of the micropyle, there is some further differentiation of the cells into what appears to be a protective layer. There is an opening in the layer at the tip of the chalazal end opposite that in the shell through which the ovular trace penetrates.

The structure and position of the pitted cells of the shell indicate the possible methods by which the developing embryo and endosperm may receive supplementary nourishment from outside the shell. It is to be noted that the neck of the nucellus extends through the micropylar end of the shell and is in contact with the obturator, while the conducting tissue of the ovular trace penetrates the chalazal end of the shell. Also, the pitted nature of the cell walls of the shell as a whole may permit some translocation or interchange of water and nutrients.

MORPHOLOGY AND PROBABLE FUNCTIONS OF THE OVULE AND ACCESSORY TISSUES

OUTER EPIDERMIS OF OUTER INTEGUMENT

The layer of cells forming the outer epidermis of the outer integument completely surrounds the anatropous ovule with the exception of the region of attachment at the funiculus and the micropylar opening. The cells are cubical or slightly rectangular, and the outer surface is eventually coated with cuticle. The main function of this layer is protection of the underlying parenchyma.

PARENCHYMA OF OUTER INTEGUMENT

The parenchyma of the outer integument is 40 to 60 cells thick by the time maximum size of the ovule is attained. The cells are irregular polygons with comparatively thin walls. The tissue is interspersed with patches of dark-staining cells with rich protoplasmic or latex content. Various types of crystals and other inclusions are found in the cells.

INNER EPIDERMIS OF OUTER INTEGUMENT

When the ovule has attained maximum size, the cells of the inner epidermis of the outer integument are elongated radially and have

comparatively thick walls. The layer is evidently quite firmly attached to the adjacent cells composing the shell and formed by the outer epidermis of the inner integument and completely surrounds them except in two regions; it forms a protective border for the micropylar canal between the apical portion of the outer integument, but it does not cover the micropylar opening; it has an opening in the chalazal tip of the ovule that permits the ovular trace to penetrate.

OUTER EPIDERMIS OF INNER INTEGUMENT

The layer forming the outer epidermis of the inner integument is the hard bony shell of the seed. There is great radial elongation of these epidermal cells to form the sclerified, pitted elements that make up the shell. The cells may be straight, slightly bowed, or knee-shaped. There are two openings in the shell. At one end of the seed claviform cells form the lower portion of the micropylar canal. At the chalazal end an opening permits the penetration of the ovular trace. The cells bordering the chalazal canal are shorter but otherwise similar in shape to the remainder of the cells forming the shell.

According to Netolitzky (*10*), the development of the outer epidermis of the inner integument into modified cells is characteristic of entire families, such as the Malvaceae, Tiliaceae, Bombacaceae, and Sterculiaceae, and is also found in the Euphorbiaceae, Dipterocarpaceae, Cistaceae, Bixaceae, Violaceae, Passifloraceae, and Thymelaeaceae. Pammel (*11*) also discussed the coils of the shell found in the euphorbiaceous seeds.

PARENCHYMA OF INNER INTEGUMENT

In the early stages of endosperm and embryo development the parenchyma of the inner integument makes up about 60 to 75 percent of the volume of the seed inside the shell. As the shell, endosperm, and embryo increase in size, the parenchyma is crushed and is almost, if not entirely, absorbed.

NUCELLUS

The nucellus may be divided into two regions with regard to probable function: (1) An upper or micropylar region above the embryo, which serves as a nucellar neck structure, extending from the suspensor of the embryo, through the micropylar canal, to the papillate cells of the obturator; and (2) a lower or chalazal region which is encroached upon and finally absorbed by the enlarging embryo sac. In both parts of the nucellus the cells contain rich protoplasm. The nucellar neck region, however, retains its rich protoplasm much longer and, furthermore, is especially rich in aleurone grains.

CONDUCTING TISSUE

The ovular trace penetrates the seed coat through the chalazal opening and branches to form a bell-shaped conducting system made up of elongated dark-staining cells that lie close to the shell but do not touch it. A thin layer of inner integument parenchyma lies between the conducting system and the shell until it is crushed by the

centrifugal expansion of the endosperm. The conducting system extends upward about two-thirds to three-fourths the length of the ovule, where it meets the inner epidermis of the inner integument. Thus a boundary is established between the nucellus and the parenchyma of the inner integument throughout the entire length of the ovule. Eventually, spiral elements develop in the conducting tissue, the parenchyma of the inner integument is partly or completely absorbed, the conducting elements come to lie adjacent to the shell and probably go to make up part of the membranelike pellicle found on the inner surface of the mature shell.

ENDOSPERM

The endosperm nuclei begin to increase in number very soon after triple fusion has occurred. At first, the free endosperm nuclei orient themselves in a single layer along the periphery of the embryo sac. This layer enlarges with the lengthening embryo sac until about the middle of June, when rapid cell division occurs, first in the region surrounding the embryo and later along the whole peripheral layer of endosperm. Cell wall formation follows almost immediately, and a solid web of tissue soon lines the entire embryo sac. The center of the embryo sac remains open, permitting extension in length of the cotyledons of the embryo.

EMBRYO

Although fertilization of the egg cell takes place within 36 hours after pollination, subsequent embryo growth is comparatively slow. The embryo attains about the 16- to 32-cell stage usually by the middle of June, but remains microscopic in size until about July 1. This delayed growth of the embryo may be caused by a lack of available nutritive materials which, up to about July 1, are required for growth of structural elements of the fruit.

The ovule attains its maximum size about July 1. The shell, developing from the outer epidermis of the inner integument by rapid radial elongation of the epidermal cells, begins to lignify at about the same time that the endosperm and embryo begin the rapid growth characteristic of these structures throughout July to late August. By this time, the internal structural development of the ovule is completed and the various parts have attained full size. The ovule parts are oriented in the manner of mature seeds. There is no further growth by cell division or enlargement.

The mature fruit consists of from one to many, usually five, seeds enclosed by a subwoody pericarp. The shell of the seed is covered with a reddish-brown dust, the remains of outer integument tissue. The seeds are somewhat flattened on the dorsal side and are slightly bulged at the ventral side of the micropylar end. The chalazal end of the seed is slightly smaller than the micropylar end. The seed coat is rough and bony and forms a point directly over the micropyle and is slightly indented at the chalazal end. The seed is oriented in the fruit so as to be parallel with the central placenta, and the micropylar end of the seed is directed toward the apex of the fruit and the chalaza toward the stem end. The kernel consists chiefly of en-

dosperm in which the embryo is embedded. The radicle of the embryo lies just below the micropylar tip formed by the seed coat, and the two flat cotyledons are oriented in the center of the seed so as to parallel with the wide axis of the seed and at right angles to the placenta.

The mature seed, when dry, is seldom filled completely. A space is usually found between the cotyledons extending in length from the plumule primordia to the ends of the cotyledons and in width from margin to margin of the cotyledons. The size of the space depends upon the amount of endosperm filling the seed.

SUMMARY

The primordia of the tung ovule develop in a manner typical of the normal anatropous ovule, with the exception of the nucellus, which protrudes through and beyond the micropylar opening formed by the two integuments.

The megaspore mother cell is hypodermal in origin. Two transverse divisions produce a row of four megaspores, of which the outer three degenerate. The chalazal megaspore enlarges and divides to form two nuclei, one orienting itself in the micropylar end of the embryo sac and the other in the chalazal end. These two nuclei then divide, thus producing two micropylar and two chalazal nuclei. The four nuclei then undergo a final division, producing four nuclei at each end of the sac. One nucleus from each group then migrates to the center of the sac, thus forming in the conventional manner a normal eight-nucleated embryo sac, consisting of an egg and two synergids in the micropylar end, three antipodals in the chalazal end, and two polar nuclei at or near the center of the sac.

The egg nucleus is fertilized between 24 and 36 hours after pollination. Synergids degenerate early, sometimes before fertilization. Antipodals also degenerate at the time of fertilization or soon after.

The fertilized egg does not immediately begin to divide but rests for a period of 1 to 2 weeks.

Embryos collected 2 to 4 weeks after pollination were found in the 8-cell stage. At 6, $7\frac{1}{2}$, and 10 weeks after pollination, most embryos were in the 32-cell stage. There is a short rest period at the 32-cell stage during the latter part of May or early June. Further divisions may occur throughout June, but rapid cell division does not begin until after July 1, when cell division and consequent increase in embryo size are rapidly accelerated. Full size is usually attained late in August.

Endosperm development was observed to begin with the first division of the $3n$ fusion nucleus in material collected 52 hours after pollination. Subsequent divisions occur regularly but relatively slowly, forming a single layer of free endosperm nuclei around the periphery of the embryo sac until about mid-June, when simultaneous cell divisions begin and cell walls are formed. Rapid endosperm development is concurrent with rapid embryo growth, and the seed is usually filled with endosperm by late August.

Morphological details of the formation of the shell, micropyle, pellicle, and chalaza are discussed in connection with their respective roles in the physiological functions of the ovule and accessory tissues.

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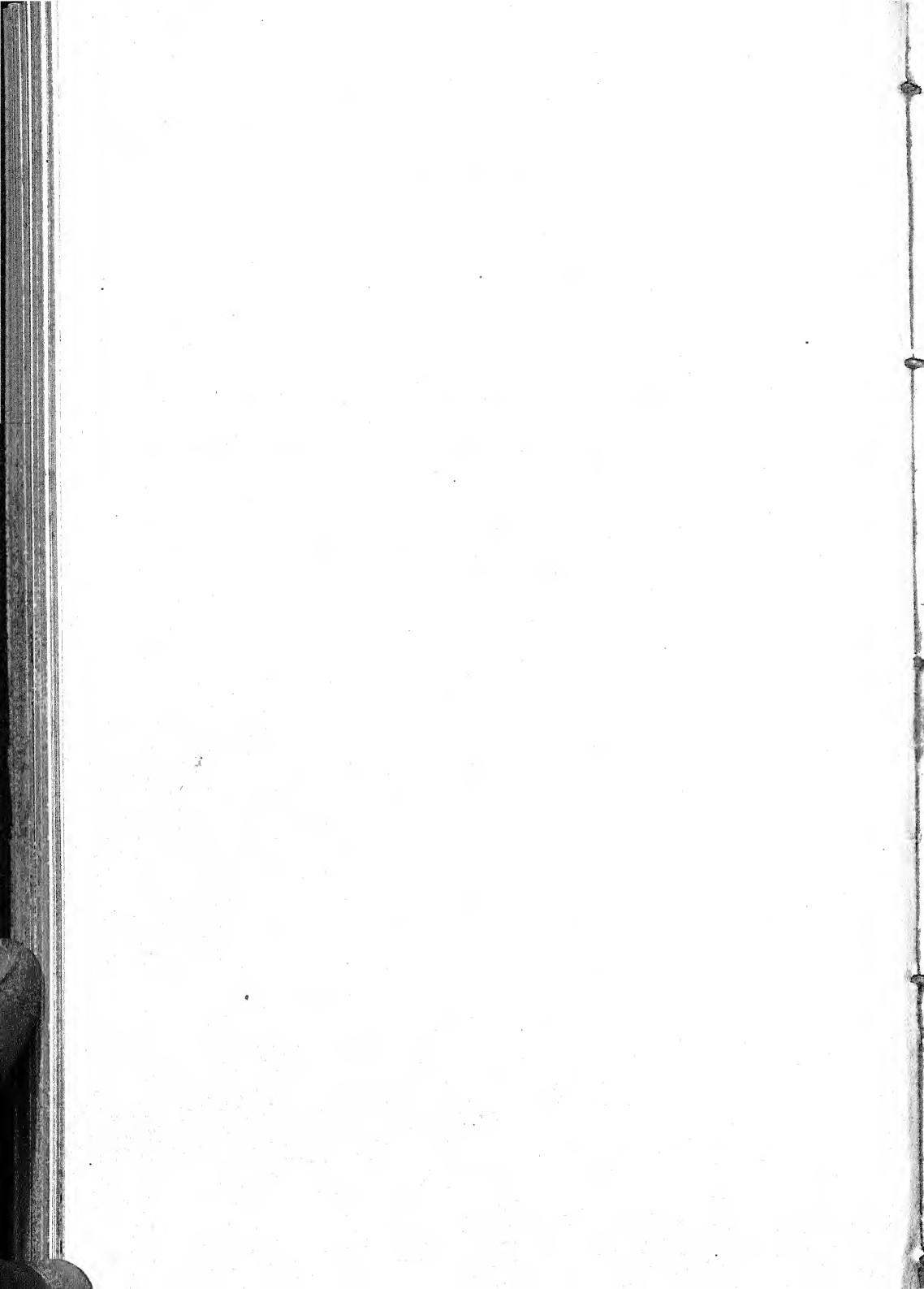
FILM FORMATION AND STRUCTURE OF SOME OIL EMULSIONS [1]

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INTRODUCTION

Incident to the development of certain oil emulsion sprays designed primarily to reduce water losses from leaves, stems, and fruits, studies have been made of certain characteristics of the films formed by different emulsions. This article reports observations on variations in film structure, as influenced by (1) certain changes in the composition of the emulsion, (2) changes in method of application, and (3) differences in the character of the surface to which they are applied.

MATERIALS

The spray materials used are divided, somewhat arbitrarily, into three groups.

Group 1.—Oil-in-water emulsions, represented by a series developed by the section of agricultural chemistry of the Michigan Experiment Station for the purpose of reducing the rate of water loss (transpiration) from plant foliage. These emulsions were prepared from plant oils of quick drying properties, and include various mixtures of linseed, soybean, and castor oils. In addition, small amounts of ammonium salts of fatty acids and proteins, together with bentonite, form the emulsifying systems.

Group 2.—Commercial oil sprays which are used as insecticides. These consist chiefly of more or less refined petroleum oils dispersed in water. The emulsifiers are for the most part trade secrets. The patents of the Standard Oil Company declare as emulsifiers for "Dendrol" a rosin or fatty acid soap (U. S. 1,785,451); for "Stanolind" glyceryl naphthenate (U. S. 1,949,799); and for "Superla" an oil-soluble hydroxy ester of organic acids (U. S. 1,949,798).

Group 3.—Certain miscellaneous materials.

METHODS

PREPARATION OF FILMS

White glass, extra thin, microscope slides were used as surfaces for the formation of films. They were thoroughly cleaned, kept in absolute alcohol, and then dried and polished before being used.

The spray materials were applied to the slides in different ways in order to determine the effect of the method of application on the

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structure of the film.   The materials were applied with (1) an ordinary hand atomizer; (2) a high-pressure spray pump; and (3) by placing a drop of the emulsion on a slide and spreading it evenly and thinly by drawing another slide over it.   No essential difference in structure of films was observed as a result of the different methods of application.

Under both (1) and (2) the vertical exposure of slides to the spray, as compared with their horizontal exposure, was tried.   This produced a difference which, while not fundamental, should be mentioned. The downward flow of the spray material upon application to the slides held in vertical position resulted in very thin, more or less imperfect films on the upper part of the slides and thicker, more or less distorted films, with some peculiarities of structure, over the lower part.   This effect will be discussed and illustrated later.   Heavier coatings of the spray materials were obtained by successive applications to the same slides after their former coatings had dried and become solid films.   Applications of the different materials were made upon slides which had been previously coated with different film-forming substances: gelatin, isobutyl-methacrylate diluted in xylol, agar-agar (3 percent), and paraffin (melting point 40° C.).

In one series of preparations the lower sides of the slides were coated over and blackened with india ink.   The aim was to reduce, if not eliminate, the reflection of light from the glass.

Only freshly prepared materials were used, and applications were made within 1 or 2 hours after their preparation.   The different emulsions were always thoroughly agitated before they were applied.

The dry oil films formed by the different methods of application, except on vertically exposed slides, were uniform over the contact surfaces, firm in their cohesive and adhesive properties, and definite and observable in their microscopic structural features, with respect to all the materials of group 1 (oil-in-water emulsions, prepared in the Michigan station chemical laboratory).   Some of the materials of group 2 (commercial oil sprays) used as insecticides formed the same type of film as those in group 1, while others formed a different type of film, which is illustrated and discussed later in this report.

The slide preparations were stored in slide cases at room temperatures.   Kept in this manner, they remained unaltered for considerable periods of time, at least as long as 8 months.

STAINING OF FILMS

Two methods for use with the materials of group 1 were devised and found satisfactory.   In the first, the oil emulsion was in a concentrated form, requiring a dilution with two parts of water before its application.   The water used for dilution was colored with 0.2 percent Nile blue and then filtered before its addition to the emulsion. After application, and upon drying, the oil phase remained colorless and the aqueous phase blue.   All attempts at counterstaining the dried films were unsatisfactory.   In the second method, the two phases of the emulsion were stained separately before being mixed: the oil with 0.15 percent scarlet red, the aqueous system with 1 percent Tieman's blue.   Mixing the two in the required proportion gave a reddish-blue emulsion, which, upon being applied and allowed to dry, retained the colors of its component parts—red oil globules, blue continuous phase.

In regard to the materials of group 2, which were obtainable only in prepared form, staining of the dried films on the slides was made possible by the use of 1 percent aqueous Nile blue solution. The excess of stain was removed by washing in distilled water. This left the continuous phase blue, the dispersed phase colorless. Attempts with counterstaining, e. g., with scarlet red in 70-percent alcoholic solution, served only to increase the difficulty of clear differentiation between the two phases.

EXAMINATION OF FILMS

Observations were for the most part limited to two principal features: (1) The course of film formation during the process of drying, and (2) the film's structure after it had dried and become fixed. Various microscopic techniques were used in these studies.

The instrument used was a Zeiss binocular microscope, with magnifications arranged at 120 ×, 300 ×, and 600 ×. The source of light was a 50-watt filament bulb, placed behind a blue glass screen. Certain variations in the direction of illumination, size of aperture, length of focus, and so forth, were employed as necessary. Furthermore, all the preparations were also examined with the aid of "Ultrapak" equipment, in order to secure vertical illumination, with an arc light as its source.

The limitations of the microscope for such studies are recognized. It does not bring under observation the elemental units and the arrangement of such units in the basic structure of emulsion films. For this purpose, resort must be made to such measurements as surface tension and potential and to employment of the ultramicroscope. However, despite their limitations, microscopic examinations of emulsion films yield important information about them.

Bütschli (*4*),[3] from microscopic observations of colloidal materials, reported a "honeycomb" or "alveolar" structure for them, and assumed this type of structure to be characteristic of both living and nonliving matter. Copisarow (*5*) observed "periodical" structures with colloids, and also "honeycombs" with dehydrated emulsions. Friedel (*6*) describes several different types of structural forms of emulsions: rods, polygonal areas, conic and nuclear configurations. He assumed that this diversity of form depended upon temperature and the degree of dilution of the materials he studied.

When emulsions are properly applied to receptive surfaces and allowed to dry thereon, their more elemental units coalesce into larger units (aggregates), and these aggregates further unite into more or less orderly and characteristic configurations. Then these configurations are microscopically observable and describable.

By studying the film structure in terms of these configurations it is possible to obtain information on the orientation and distribution of the coarser structural elements as well as on the coarser structures of the films. The final result from efforts to observe and comprehend the behavior of these aggregated particles may be, as some workers contend, a true and useful way to arrive at an understanding of the elemental and invisible film structure.

A Leitz Panphot Universal Camera was used to make the microphotographs of the prepared slides. Magnifications obtained by

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[3] Italic numbers in parentheses refer to Literature Cited, p. 251.

means of this equipment were about 500× and 1,000×. Approximately 200 microphotographs were made of the slides at hand. Reproductions showing typical conditions are presented in the following section.

RESULTS

MATERIALS OF GROUP 1 (OIL-IN-WATER EMULSIONS)

THE FORMATION OF FILMS

When emulsions of oil in water, which include many spray materials, are spread over a receptive surface and allowed to dry, the final result is a solid, fixed film. Various physical (evaporation of free water) and chemical (oxidation, polymerization, etc.) phenomena are involved and interwoven in the drying process.

The transformation from liquid to solid is not instantaneous. Time intervenes during which the progress of the change, which results in the formation of larger and larger particles and their aggregation into permanent configurations, may be observed under the microscope.

Figures 1 and 2 illustrate the general film-forming behavior of oil-in-water emulsions. They show successive stages in the drying process from the application of the emulsion (7.5 percent oil, 1 percent bentonite) to the slide, up to the final completely dried and permanent film.

Caught in figure 1, *A* (emulsion immediately after application) is a curving streamlike motion of the water which carries the dispersed oil globules passively along with it. Variation in size of the globules was from the limit of visibility to 16 microns in diameter. The smallest ones (diameters up to 4 microns) showed Brownian movement. The larger globules are relatively few in number. They not only vary in size but also in transparency, from being wholly transparent to considerably clouded. Adherence of the globules, especially of smaller ones to form larger ones, is conspicuous. This indicates a force of attraction which is high, since the interfacial tension between like molecules of oil is low (*1*, *2*). It presages the coalescence of globules to form globular units of larger size, which have the smallest possible surface areas for the volumes concerned. Other globules may be kept from contact with each other by their electric charges and mutual repulsion.

Figure 1, B, shows the same coating after the lapse of 1 minute. Streaming continues, but is more curvilinear in direction. The smaller globules are still being carried along in the current, but the larger ones, both singly and grouped, have come to rest. The number of visible globules has increased. Possibly not all of these are composed of oil alone. According to Adam (*1*) they may represent the various combinations of the phases of the emulsion, in this case, oil, protein, bentonite. Their emergence out of the complex continuous phase is perhaps mostly marginal, since at the edges of the film the globules behave like molecules when the surface is contracting. That is, they continuously move inwardly because of not being balanced in their inward-moving tendency by outward-moving globules of the same number (*1*).

The phenomenon of "multiple emulsion" (*13*) is apparent in several of the larger globules. This consists in a globule of the dispersed phase being an emulsion by containing, within itself, smaller globules

of the other phase, or phases. Adam (*1*) has suggested that this occurs only when at least two different emulsifying agents are present.
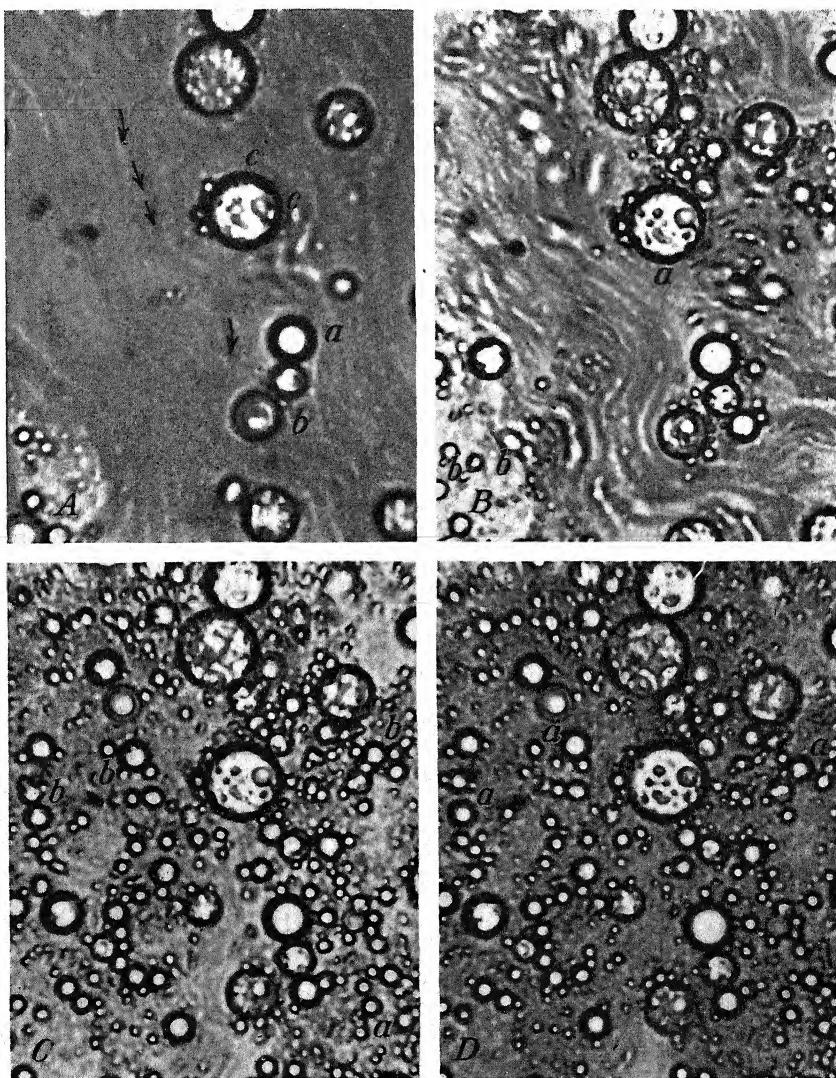
FIGURE 1.—Drying stages of a spray film of an emulsion of 7.5 percent oil, 1 percent bentonite. *A*, Film in the fresh state immediately after application on glass. Arrows, Streaming motion; *a*, transparent oil globule; *b*, clouded globule; *c*, multiple emulsion. *B*, Film after 1 minute; largest oil globules have come to rest: *a*, Multiple emulsion; *b*, coalescence. *C*, Film after 3 minutes: *a*, Coalescence of small globules; *b*, attraction of globules. *D*, Film after 5 minutes; streaming motion has practically ceased: *a*, Repulsion of one globule by another. All ×1,000.

This was substantiated in the present study by the observation that "multiple emulsion" occurred only with the materials of group 1, in which the emulsifying system contained bentonite in addition to fatty

acids and proteinates. The tendency for newly emerged globules to cluster together and the coalescence of some globules are shown as being under way.

Further developments are shown in figure 1, *C* which illustrates the film after the lapse of 3 minutes. The solution is still motile, but only slightly so. Definite streaming is confined mostly to a narrow channel in the center of the lower half of the figure. In, or out of this moving area small globules are still coming into visibility. The number of globules is considerably greater than that shown in fig. 1, *B*. Many which are only vaguely visible and others not as yet visible in figure 1, *B*, have appeared definitely. The phenomena of attraction between globules, and of their coalescence, are more abundantly illustrated in figure 1, *C*, than in *B*.

Figure 1, *D*, shows the film after 5 minutes of drying. Perceptible motion has virtually ceased. It continues, however, to some degree in the two or three lighter (less dry) patches which are readliy distinguishable.

Attention is directed to what appear to be cases of globular repulsion. This is evident from a comparison of *C* and *D*, for the groups of globules concerned in the action. This tendency exists and is operative, in contrast to the stronger and more general movement towards adherence and the ultimate coalescence of the dispersed globules.

Loss of motility is a function of the rate of drying, and this rate is more or less contingent upon the temperature, the particular composition and concentration of the emulsion, and the thickness of the film. For the materials of group 1, under the varied conditions of the observations made, the minimum time for loss of motility was 5 minutes, with an occasional maximum of 10 minutes.

During the 5 to 10 minutes immediately following the cessation of perceptible motility, certain changes take place. A description of these changes, as observed and illustrated in figure 2, is briefly as follows: Very small gaps were seen to open in the stationary globules. These openings appeared suddenly, taking no longer than a so-called "split second." The gaps closed as rapidly as they opened, and left behind no marks of their occurence. A hypothetical explanation of this phenomenon could be that of an excessively high capillary pressure exerted by entrapped air bubbles, directed towards the centers of the globules. This was antecedent to a depression of the spherical walls of the globules and led to the immediate disappearance of the air bubbles. Watching this, one might think that these bubbles were being swallowed by the surrounding fluid. Actually, however, they may have been bursting, as a consequence of increased surface pressure which was due to the compression of the film by increased drying.

Subsequently, the coalescence of the smaller globules, on a rather general scale, was observed. The larger oil globules became almost instantaneously deformed and showed radial protrusions which broke up their strictly spherical shapes (fig. 2, *A*). Presumably, at least, this is a manifestation of an increased pull on these globules because of augmented surface tension due to increased evaporation of water from the film. Upon the completion of this stage, the spherical oil globules have changed into areas with outlines which are more or less angular (hexagonal tendency) and the consequence is the typical "honeycomb" structure shown in figure 2, *B*, *a*, as described by Bütschli (*4*).
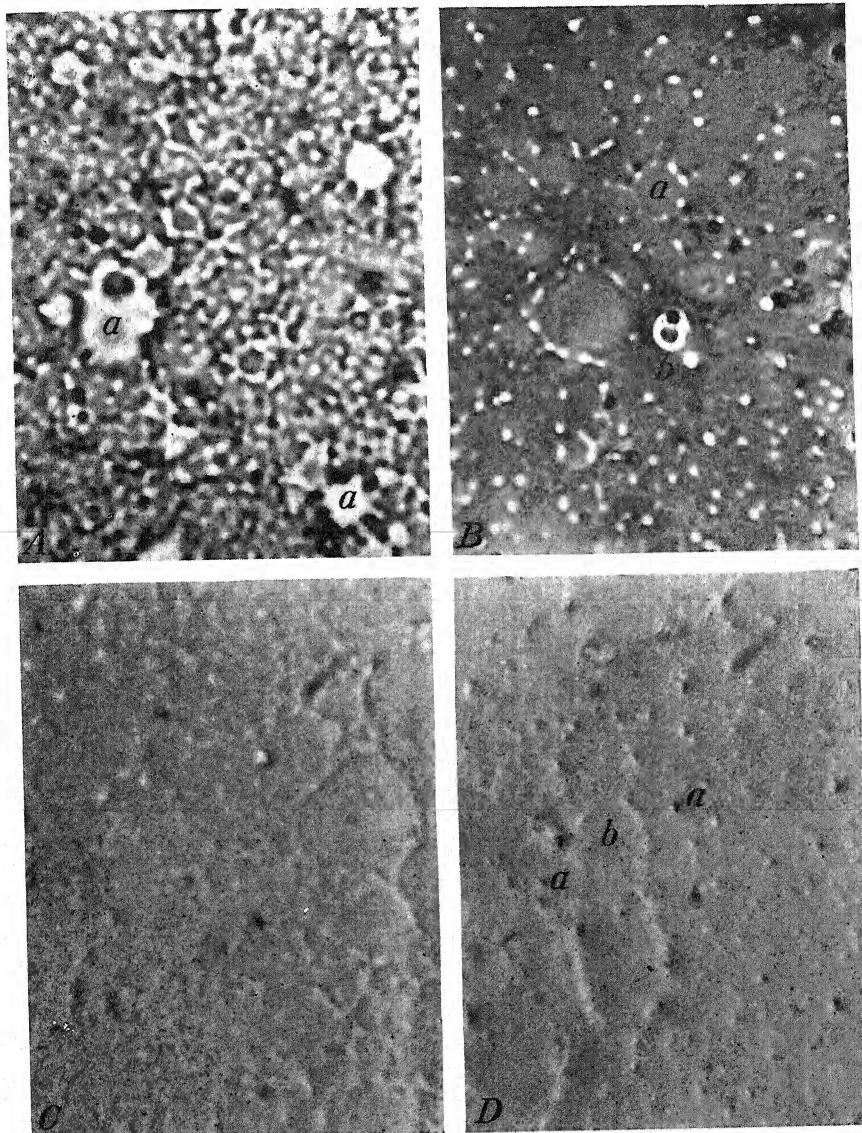
FIGURE 2.—Progressive film formation, oil-in-water emulsion. *A*, Film after 8 minutes of drying: *a*. Amoeboid form of a drying oil globule. *B*, Latest stage in formation of solid film; film after about 10 minutes of drying: *a*. "honeycomb" area; *b*, oil globule not yet dried. *C*, Film completely dried, under ordinary focus. *D*, Film completely dried, under high focus: *a*, elevated nodules; *b*, crateriform depression. All × 1,000.

The explanation Seifriz (*14, p. 197*) gave for the formation of hexagons of drying mud may also explain the emulsion systems under consideration:

. . . When a body is in a state of strain, as is true of drying mud, the lines of force set up are at maximum stability when three emanate from a point so as to form three angles of 120°. It is for this reason that hexagons are formed when mud dries. . . . The tendency of liquids and semifluid (plastic) systems to adjust themselves to or toward this condition, accounts for the prevalence of the hexagon . . . angle.

It will be noted in figure 2, *B*, that the apparently blank, hexagonal areas are outlined and somewhat connected by chains of brighter spots which appear to be the remaining aggregates of crowded particles from dried globules. These chains form sheaths around the blank more or less hexagonal areas. If their bright spots are not tightly packed together as in a string of beads, but some distance remains between them, they are attached to each other by rodlike bridges. These spots might be assumed to be mere optical artifacts. That they are not such is indicated by the observation that at and above all magnifications of $600 \times$ they passed in all directions through the film, under both transmitted and incident light, with all variations of focus and of size of aperture.

Figure 2, *C*, shows a completely dried and permanently fixed film, under ordinary focus. The honeycomb appearance of structure persists. The light, circular spots, seen serving as borders and connecting lines for the larger blank areas in *B* as such have mostly disappeared. The rims of surrounded areas and the lines connecting them now appear to be solid, with a rough, nodular, uneven aspect.

Figure 2, *D*, shows a completely dried film when observed under high microscopic focus. Two structural elements are most conspicuous: pimplelike, elevated nodules and crateriform, polyhedrous depressions whose bordering rims are characterized by elevated, linked-together nodules. Thus, the final pattern appears to be that of a kind of mosaic, with the residual, polyhedrous figures of the dispersed oil globules outlined, and connected more or less completely, by the dried aggregates of the emulsifying agents. These same agents, in a dried state, constitute the basic, continuous, "warp and woof," so to speak, of the solid film coat.

VARIATION IN THE COMPOSITION OF THE EMULSION

The process of film formation so far described and illustrated is based upon observations on a single one (7.5 percent oil, 1 percent bentonite) of the several materials of group 1. This representation is fundamentally typical for the group. However, certain superficial alterations, worthy of attention, occurred when the proportional amounts of the constituents of the emulsion were varied.

In general, the effect from proportionally less oil and more of one of the solid components in the emulsion (3 percent oil, 3 percent bentonite) was, as shown by figure 3, *A*, that of a finer network and a denser film structure. With proportionally more oil and less of the solid constituent (10 percent oil, 3 percent bentonite), the result, as shown by figure 3, *B*, was that of a film less dense in character. The hexagonal areas are more numerous and larger in size, and the interstices between them are more of the nature of peripheric seams, or chain linkages.

<center>SUPERIMPOSED FILMS</center>

Repeated applications of the same material were made to previously applied and dried films.   The results are illustrated in figure
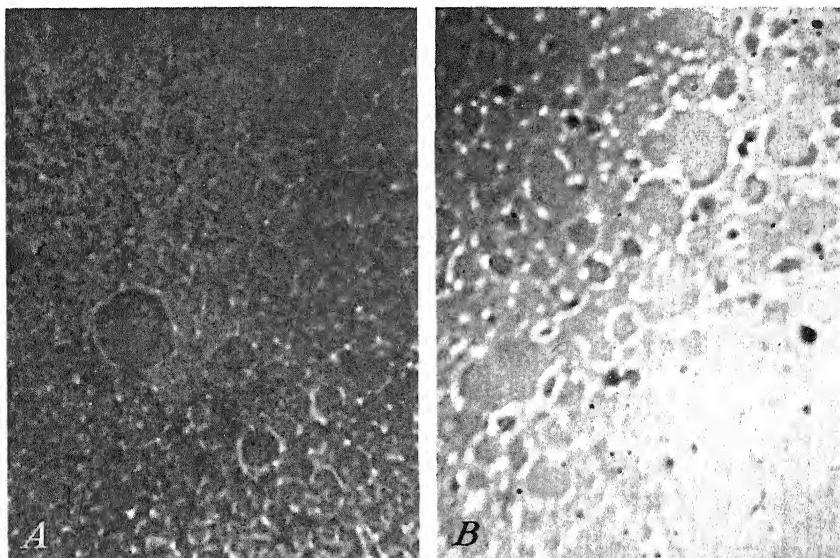
FIGURE 3.—Variation in the composition of oil-in-water emulsion. *A*, Emulsion film of 3 percent oil, 3 percent bentonite, 1 coat. *B*, Emulsion film of 10 percent oil, 3 percent bentonite, 1 coat.   × 1,000.

4.   *A* and *B* of this figure show effects secured by two and four applications of an emulsion composed of 3 percent oil and 3 percent bentonite.

Progressive thickening and densification of the film is obvious. Distortion and destruction of the original pattern is equally obvious. Adherence between the successive films to form a final film with greater thickness was perfect, except for a peculiarity which is noted in figure 4, *B*. Dark spots appeared here and there in the film. These might be interpreted as the effect of air bubbles entrapped between the successive layers and not discharged in the process of attaining final equilibrium between these layers.

Figure 3, *B*, shows, in conjunction with figure 4, *C, D*, a series of one, two, and four coats of an emulsion composed of 10 percent oil and 3 percent bentonite. Clearly, the effect of successive coats in terms of densification of the film and distortion of its pattern is less pronounced. Furthermore, the presence and survival of encircled dark areas, such as shown in figure 4, *C*, was always observed.

<center>THE EFFECT OF DIFFERENT SUBSTRATA</center>

The wetting properties of certain spray materials on different types of plant surfaces and on paraffin-coated slides has been observed by O'Kane and his coworkers (*7, 8, 9, 10, 11*). Their studies showed clearly that the physicochemical properties of both the spray ma-
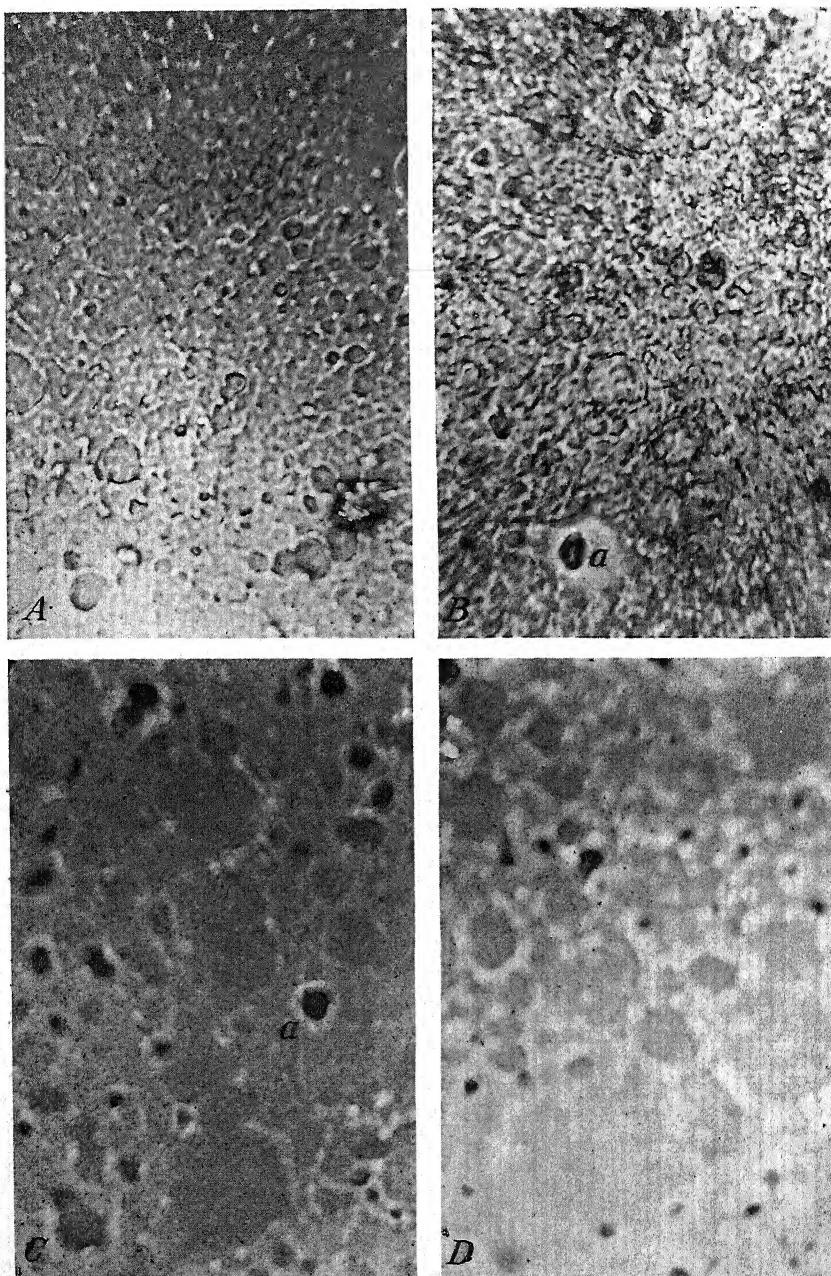
FIGURE 4.—Superimposed films, oil-in-water emulsions. *A*, Two coats of an emulsion of 3 percent oil, 3 percent bentonite. *B*, Four coats of an emulsion of 3 percent oil, 3 percent bentonite; *a*, presumably an air bubble. *C*, Two coats of an emulsion of 10 percent oil, 3 percent bentonite; *a*, oily spaces of both layers superimposed. *D*, Slide coated four times with emulsion of 10 percent oil, 3 percent bentonite. All × 900.

terials and the substrata to which they were applied need to be considered if an understanding of the formation and the visible structure of spray films is to be obtained. The molecular movement, in the liquid state, of an emulsion (spray material) may determine the nature of the final orientation. On the other hand, the fixed molecular orientation of the solid surface, or substratum, with its existing surface energy influences the liquid's adhesive possibility and its tendency for orientation during the process of its drying and becoming permanently filmlike in character.

Different substances were used for coatings on glass slides in order to secure a variety of substrata for the application of one of the oil-in-water emulsions—7.5 percent oil, 1 percent bentonite. These substances included gelatin, isobutyl-methacrylate, agar, and paraffin. Basic films (surfaces) of each of these four substances were produced by spreading the substance over the slide in a thin layer and allowing it to dry and solidify thereon, at room temperature.

With the exception of paraffin, continuous, transparent, microscopically structureless films were obtained. Paraffin is not, as the others are, an optically isotropic substance; a paraffin film has a distinctly patterned structure. This properly places it among the materials of group 3. It will be illustrated and discussed in a later section.

Figure 5, *A*, shows a dried film of the emulsion on a surface of gelatin; *B*, a film of the same emulsion on a surface of isobutyl-methacrylate, and *C*, the same emulsion on a surface of agar. These films may be compared with those in figure 2, *B–D* (dried films of the same emulsion on plain glass surfaces). The three films in figure 5 differ among themselves, and none is identical in aspect with those on the glass surface (fig. 2, *B–D*). However, alterations due to the substrata are only superficial; the fundamental honeycomb structure persists and prevails, and shows merely changes in the proportional relationships of its basic features.

In figure 5, *A* (gelatin surface) the honeycomb structure is notably irregular and dense. The hexagonal areas are numerous, mostly small in size, crowded together to the point of overlapping, and are in general neither sharp in outline nor clear in their contents. Their intermixture with the emulsifying agents is pronounced. Evidently certain areas concentrate more of the liquid than others, and strands of compact lines are formed. This possibly is indicative of contact angles of larger sizes, which, in turn, are antecedent to decreased spreading potentiality of the emulsion.

On a surface of isobutyl-methacrylate (fig. 5, *B*) the openlike spaces of the film are especially distinct and large in size. They are relatively few in number and separated in their locations. The framework of meshes, consequently, composes the larger part of the entire film, and has quite distinctly spotted chains for its connecting strands. Apparently the substratum has good wettability. A high tensile stress, operating at and inwardly from the periphery of the film seems to have expanded the oil globules, while the aggregates of the rest of the material become localized and concentrated in the matrix of the film.

The agar surface (fig. 5, *C*) seems to have exercised the most pronounced influence on the spread and expansion of the emulsion on the film. The adhesive force of the solid agar surface for the

emulsion appears greatly to exceed the cohesive forces of the ingredients of the emulsion within itself. Consequently, the open
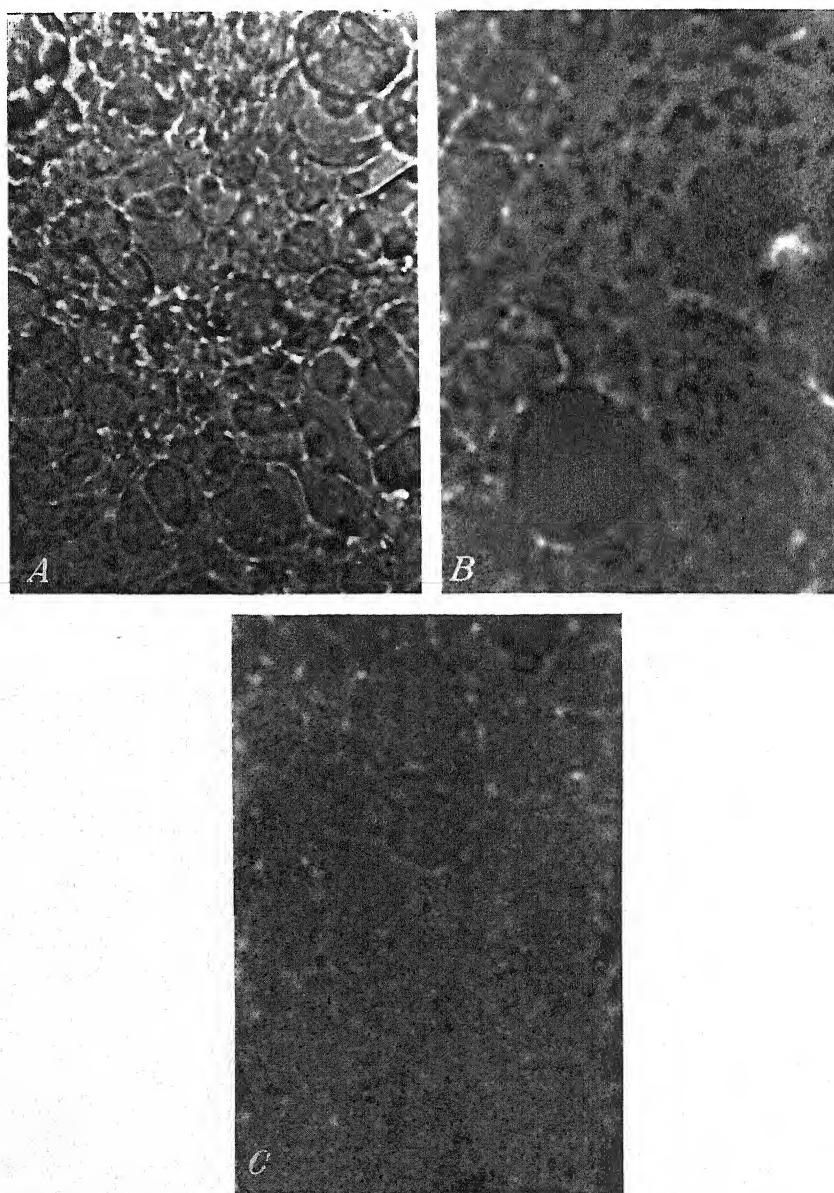
FIGURE 5.—Effect of different substrata with oil-in-water emulsions. *A*, Dried film of oil-in-water emulsion (7.5 percent oil, 1 percent bentonite) on gelatin surface. *B*, Dried film of same emulsion on a surface of isobutyl-methacrylate. *C*, Dried film of same emulsion on agar surface. × 1,000.

spaces (dried oil globules) are relatively large, numerous, close together and almost coherent, and separated only by their respective

rims, the rest of the system being minor in area and spongelike in appearance.

In general, then, the emulsion films superposed on these three types of surface varied in detail, with respect to the relative distribution of the materials of the emulsion, but showed the same fundamental honeycomb pattern that they exhibit when produced on a plain glass surface. They apparently responded individually to the decisive factor of the proportion between the perpendicular and the lateral forces of adhesion which are operative in film formation on different types of surfaces.

MATERIALS OF GROUP 2 (COMMERCIAL OIL SPRAYS)

The materials of group 2, the commercial petroleum-oil sprays, included both dormant- and summer-spray oils. The former contain higher concentrations of oils than the latter. As a group, these emulsions differ from the materials of group 1 principally in that they contain somewhat less effective emulsifying and stabilizing components. Hence, although possessing sufficient spreading and wetting ability for ordinary use as sprays, they are relatively unstable in comparison with the materials in group 1. This lack of stability causes them, when applied, to spread rapidly and to break readily. Far from being considered detrimental to their efficiency as insecticides, this property of quick spreading and breaking is said to be advantageous (U. S. Patent No. 1,949,799). The results from trials with six of these commercial materials are illustrated in figures 6 to 9.

Figure 6, *A*, shows a prepared film of "Standard Aphid Spray Oil," in 2-percent concentration with water, on a glass slide surface. Shiny, circularly outlined spots appear which are irregularly distributed throughout an opaque isotropic matrix. In some places the bright globules are in close proximity and then appear to be arranged in short chains like strings of beads. In such places the film appeared to be somewhat thicker than elsewhere.

Figure 6, *B*, shows a film of "Stanolind," 4-percent concentration, on a glass surface. The bright spots are more numerous than in the film formed by a 2-percent concentration of Standard Aphid Spray Oil (fig. 6, *A*), and they are more generally intermixed and crowded together. They give some evidence of a faintly defined structure that is reticulate in character.

Figure 6, *C*, shows a film of "Dendrol," 4 percent concentration. This film differs from those shown in *A* and *B* principally in the greater number and smaller size of its spots and the fact that they are all mostly transparent. Close scrutiny reveals that these spots tend to form chains of from 2 to 10 units and that these chains tend somewhat towards the formation of a netlike pattern.

Figure 6, *D*, shows a film formed by a 1-percent concentration of "Superla." This spray yields a coarse emulsion, in a reverting state (passing from the oil-in-water to a water-in-oil system). The interfacial tension is thereby reduced to a minimum. The aqueous phase shows no tendency to form spherical droplets, but appears in large, irregular-shaped drops. Some of these contain small oil globules, indicating the presence of a "bimultiple" system (*13*). The dark outlines of the water spaces are the optical consequence of different refractive indices between the two zones. The inner line is lighter, which indicates that the external phase has the higher index.

Figure 7 shows films of the material known as "Free-Mulsion," 4-percent concentration. *A* shows the lacelike pattern of the film which formed on the lower portion of a vertically suspended slide.
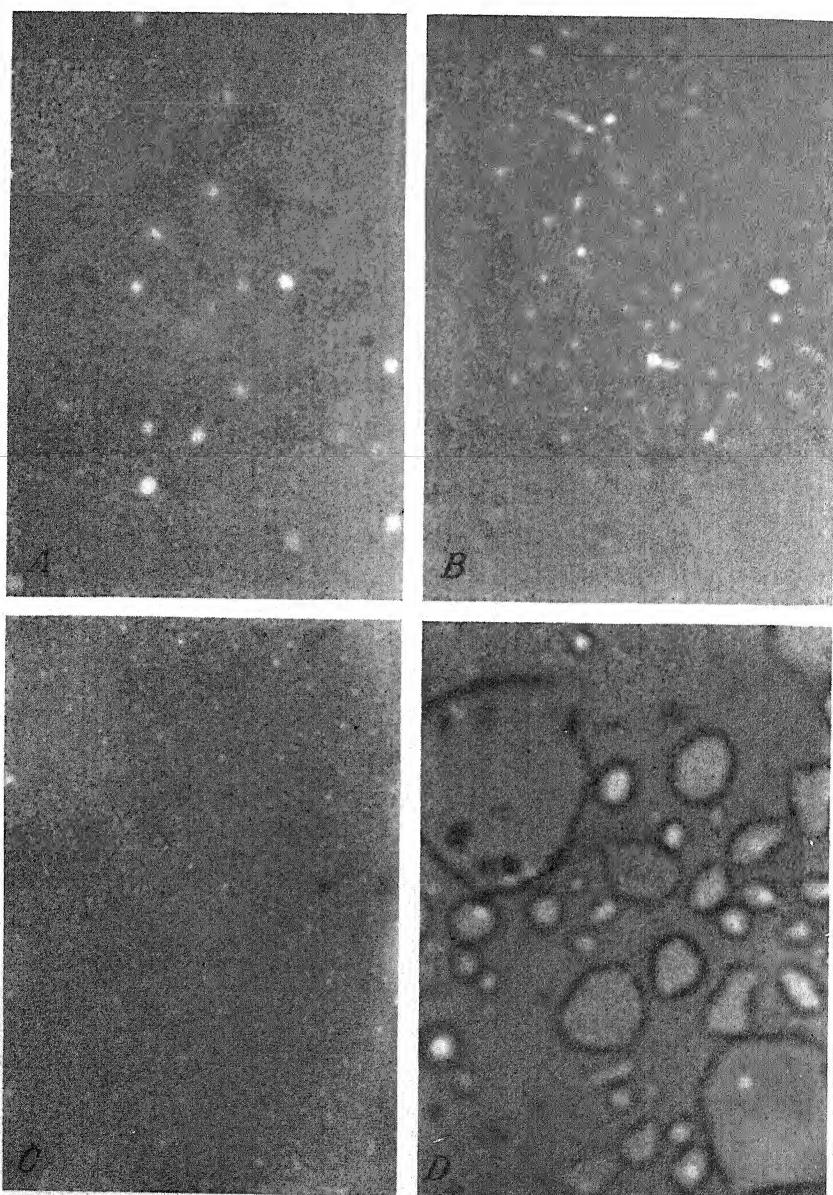
FIGURE 6.—*A*, Film of Standard Aphid Spray Oil on glass. × 1,000. *B*, Film of Stanolind on glass. × 1,000. *C*, Film of Dendrol on glass. × 450. *D*, Film of Superla on glass. × 450.

The emulsion is in an initial state of demulsification. Its surface tension is greater than that of the previously described material

Superla, but the adhesion between the liquid and the glass was too weak to give an even film. The upper part of the film (not illustrated) was thinner, with only a few small and scattered globular areas in its composition. The larger defined areas shown in *A* were formed
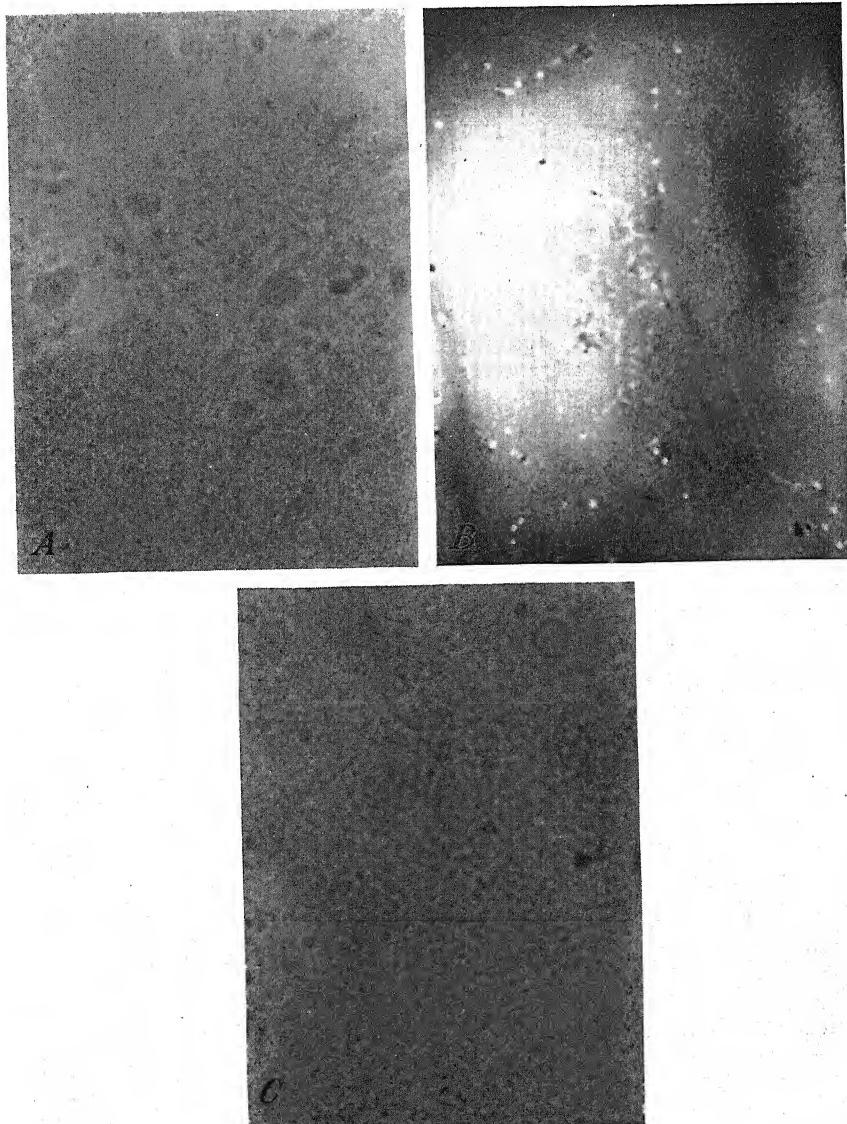
FIGURE 7.—*A*, Film of Free-Mulsion on vertically exposed glass slide.   × 1,000.
B, Film of Free-Mulsion on horizontally exposed glass slide.   × 450.   *C*, Film of Free-Mulsion on gelatin-coated, vertically exposed slide.   × 1,000.

by droplets of oil assembling in pools, coalescing, and losing their separate identities.

Figure 7, *B*, shows a film of the same material, which was formed on a slide horizontally exposed to the spray. A difference due to the

position of the receptive surface, with this quick-breaking emulsion is noteworthy. There appears to be an aggregation of particles, with these aggregates alined in the form of filaments, which link together to encircle the globular areas and so produce an elongated, netlike pattern. In the areas outside of the patterned ones appear only scattered spots with little or no contact. There were certain channels of flow while the material was in the fluid state, and these elongated patterns tended to develop in and out of these currents of liquid motion. Especially at the border of the streaming fluid the aggregates are deposited densely enough to become oriented into filaments.

Free-mulsion was applied also to slides bearing a gelatin film on their surfaces. The results are shown in figure 7, *C*, when the exposure of the surface to the spray was vertical. The contrast with *A* (also vertical exposure) is interesting. Obviously, the adhesive force between the gelatin surface and the emulsion is strong. The contact angles apparently are small and decisive. Despite the vertical exposure of the surface and the down pull of gravity for the freshly applied material, the coating remains almost uniformly thick over the entire surface. Furthermore, it comes to have a continuous structure that is similar to the honeycomb pattern formed by the oil-in-water emulsions of group 1.

Figures 8 and 9 show the results obtained with a material called Summer-Mulsion, 1-percent concentration.

Figure 8, *A*, represents a film of this emulsion on a vertically exposed slide. In the wake of the downflow of the solution there appears a mass of small globular spots interspersed through the solid matrix. Some degree of order appears in the partial union, and even coalescence, of the globules into groups, and also into chains. Figure 8, *B*, shows a film of this material on a horizontally exposed slide. This film contrasts strikingly with that in *A* (vertical exposure of slide). Dark, nodular demarcation lines enclose circular and polyhedrous areas. These vary in size and are joined together by more or less loosely connected aggregates. The dark (black) spots may be interpreted as air pores. Figures 8, *C*, shows another place in the same film as that pictured in figure 8, *B*, but the two images are very different. That in *C* shows much similarity to the lacelike pattern produced by Free-Mulsion (fig. 7, *A*). This quality of pattern in the same film may have resulted from a condition of non-uniformity of the emulsion itself with respect to density, dispersion, or some other property. The place where the lacelike pattern appears indicates the behavior of an unstable emulsion, on the point of reverting (*12*).

Figure 9, *A*, shows the film produced by an application of Summer-Mulsion, 1-percent concentration, to a gelatin-coated slide, vertically exposed. Aside from a relatively few outlined, somewhat larger, circular areas, the aspect is that of a continuous, indefinitely figured netting, composed of dark-centered, lightly outlined spaces. The aggregated particles are mostly in direct contact with each other.

Figure 9, *B*, shows the result of applying Summer-Mulsion, 1-percent concentration, to a surface of isobutyl-methacrylate on a glass slide. The image, obtained by illumination from below, shows medallionlike forms, scattered here and there throughout the entire film.
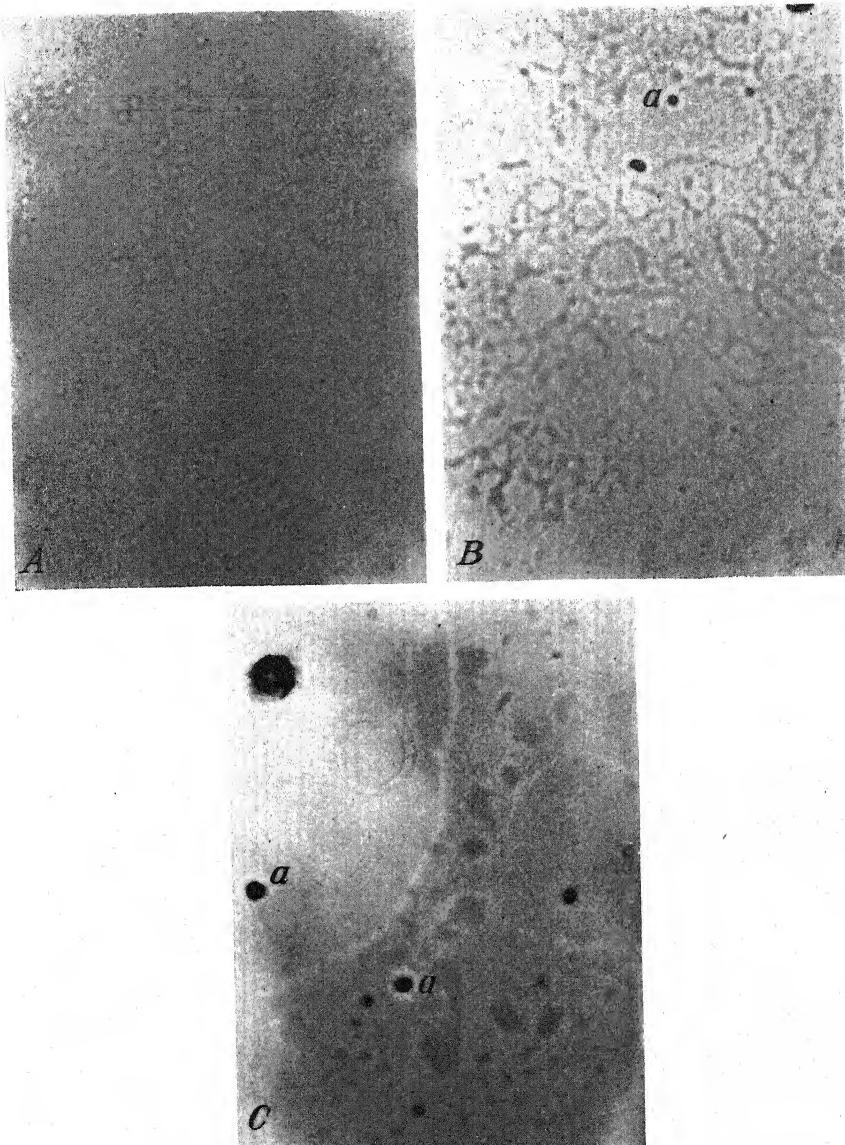
FIGURE 8.—*A*, Film of Summer-Mulsion on vertically exposed glass slide. *B*, Summer-Mulsion on horizontally exposed glass slide. *C*, Same film preparation as *B*, at another place. *a*, Apparently air pores. All × 1,000.

Careful observation of the process of the film's formation suggests the following explanation. The pattern may be a result of double film layers, originating from a breaking emulsion under certain mechanical conditions. When the emulsion breaks up into droplets, a single droplet is subjected to the divergent forces of the air-liquid and the liquid-solid surfaces and to tensions within the liquid itself. At the contact between the droplet and its substratum,

interfacial tension fixes it to the solid surface, while above its center the particles still are floating freely until evaporation lowers the level
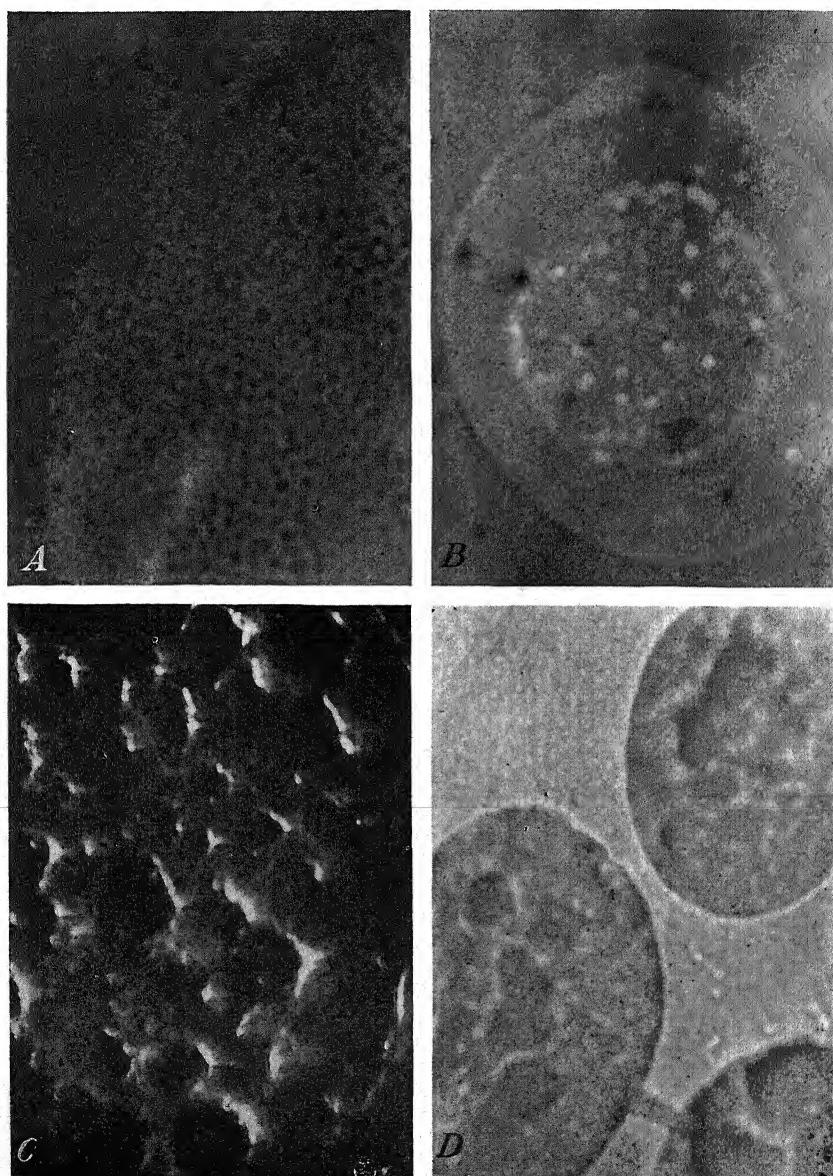
FIGURE 9.—*A*, Film of Summer-Mulsion on gelatin-coated, vertically exposed slide. ×.1,000. *B*, Film of Summer-Mulsion on a methacrylate-coated slide, showing medallionlike structure. × 450. *C*, Same film preparation as *B*, showing central area of medallion; light source from above (Ultrapak). × 1,000. *D*, Film of Summer-Mulsion on agar-coated slide. × 1,000.

and these particles are also brought into close proximity with the already more solidified lower lamella of the film. Then, as the

droplet dries still further, the particles and aggregates are pulled together into a smaller area above, while the underlying material remains circularly expanded and fixed. Upon drying, the medallion-like structure results, consisting of two concentric circular plates. The exterior one appears like a frame, outlined by a cohering chain of aggregates, with a few single scattered aggregates in the outer arch. The inner circle is likewise bordered at its periphery by a beadlike bright string of aggregates. In the center a number of structural units are visible, showing some degree of attachment and grouping. The aggregates in the inner circle of the pattern are, for the most part, loosely assembled. In certain other "medallions" in the same film, the particles of the inner circles were seen to be more closely associated and linked together, even to the point of the formation of a mostly definite structure. This structure was of the nature of hexagonally-shaped homogeneous areas, surrounded and defined by borders composed of the linked aggregates.

Figure 9, *C*, shows the central area of the medallion pictured in *B*, when observed under illumination from above. Deep-seated structure is thereby revealed, and the three-dimensional character of this structure is confirmed. The image is quite appropriate for the explanation offered by Bender (*3*) for the structural formation and character of resinous films. He detected "matchlike macroparticles" present as building stones, oriented into parallel position when the film is subjected to forces of alinement.

Figure 9, *D*, gives the pattern formed by Summer-Mulsion when spread upon a surface of agar. The medallions here are oval in shape. This may be due to the upper lamellae of the film not expanding simultaneously on its entire periphery. The medallions are embedded in an anisotropic substratum. The structure of this "deeper" portion of the film is that of a dense, tight network, which in some instances is channeled between medallions in close proximity to each other. The interiors of the medallions are, on the whole, representative of the typical honeycomb arrangement observed with the materials of group 1 (oil-in-water emulsions).

MATERIALS OF GROUP 3 (MISCELLANEOUS SUBSTANCES)

BENTONITE

Bentonite is a finely powdered aluminum silicate, the particles of which range in size from 10^{-4} to 10^{-2} microns. When suspended in water, it holds the liquid both mechanically (within its capillary pore spaces) and by adsorption, in the form of films over the surface of its particles. The particles of bentonite properly combined with other materials in an oil emulsion serve to increase the amount of the internally exposed surfaces of these materials. Consequently their interfacial tensions are lowered, the molecules of the different phases are anchored, and the presence of the bentonite has a stabilizing effect upon the emulsion. Since bentonite is an ingredient of the spray emulsions of group 1, it seemed desirable to examine its structure when applied alone to glass slides. This promised to give information with respect to its influence upon film formation and structure for the emulsions of which it is an incorporated substance.

Figure 10, *A*, shows the film of a 5-percent bentonite suspension in water, with illumination from below, and at high magnification.

The aggregates—for the most part small in size, light in appearance, mostly circular in shape—lie at different levels. They are, to a slight
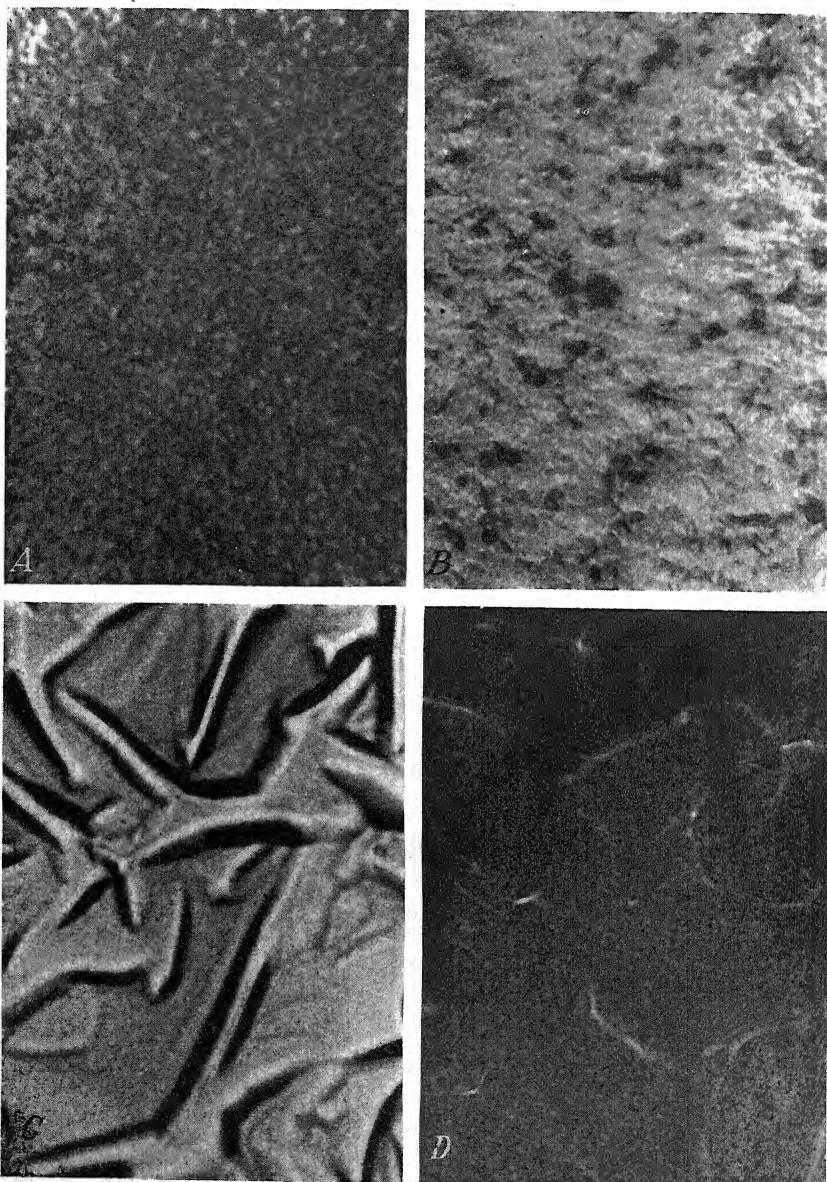
FIGURE 10.—*A*, Film of a 5-percent bentonite suspension on glass. ×1,000. *B*, Film of 3-percent bentonite suspension on glass. ×460. *C*, Film of paraffin on glass. ×1,000. *D*, Same as *C*, but at high focus. ×1,000.

extent, linked together by adhesion, and even by connecting bars, but, on the whole, they are irregularly distributed and give the general appearance of random distribution.

Figure 10, *B*, shows the film of a 3-percent bentonite suspension, under lower magnification, illuminated from above. The aspect is gross in character and the film appears to be rippled and more or less stratified. Scarcely any definitely formed (circular or otherwise) units are observable. Nevertheless, the structure is coherent, except for being interrupted here and there by fissures of various shapes and sizes, which might result from the evaporation of water.

The microscopic examinations of aqueous suspensions of pure bentonite showed their aggregates, as formed in the dry films, to be essentially like those found in the emulsions which were examined, especially those of group 1-in which bentonite was always an ingredient. However, in no pure bentonite suspension examined did the aggregates show a tendency to form a definite pattern, such as a honeycomb, lacelike, or medallionlike configuration.

PARAFFIN

Paraffin, unlike gelatin and agar, when spread in melted form as a film on a glass slide and allowed to cool and set gives a distinct pattern. Contraction from cooling is rapid and decisive. This results in a kind of buckling of the material, followed immediately by a heterogeneous twisting due to retroactive tensile forces. This in turn leads to the production of a film showing plastic configurations, which are sharp in contrast (fig. 10, *C*).

Figure 10, *D*, shows an area of the same film as that in figure 10, *C*, observed at the same magnification but at high focus. The three-dimensional character of the structure in *C*, has disappeared, but the contours of the highest level of the film's relief remain projected. Clearly, the surface of the film is uneven and rough. This condition would be expected to reflect itself in the structural formations of other materials, such as spray emulsions, should they be applied to it and dry thereon. This was experimentally tried and confirmed with the materials of both group 1 and group 2.

A paraffin layer, or film, is resistant to wetting. The molecular orientation of the substance is such that its hydrocarbon chains form a surface layer which practically covers the molecular groups that possess the greater attraction for water. The contact angles of the substance for water and certain organic liquids are as large as 105° (*1*).

Figure 11, *A*, shows a paraffin film, on glass, coated over with the film of an emulsion (group 1) containing 7.5 percent oil and 1 percent bentonite. The effect of the roughness of the paraffin surface is clearly apparent. The film is not continuously coherent, being absent from the elevated and sloping parts of the surface. Gravity, as was observed, exceeded the forces of adhesion and wettability as the state of surface equilibrium was being reached; the emulsion flowed and settled into the depressions and solidified, leaving the elevated part of the contour uncovered. Upon drying, a film formed in these sunken areas which adhered to the surface of the paraffin and the material took on a honeycomb aspect, not essentially different from that which characterizes a film of this emulsion on a gelatin or agar surface.

Figure 11, *B*, shows a film of Summer-Mulsion (group 2) on a paraffin surface. The aspect is quite different from that of figure 11, *A*, (material of group 1). No formal pattern, either locally or generally, is apparent. Because of the poor wettability of the substratum the liquid spray has no chance to form a coherent and complete film.

Puddles of the emulsion are scattered over the surface; they fill the depressions in the paraffin and their agglomerated particles settle down and solidify. Manifold configurations arise within these isolated areas, in consequence of the probably different physical forces present at the individual places to which the emulsion droplets are confined. The structure of these film fragments consists mostly of adhering globular oil bodies and varies in density. Coverage of the paraffin surface by the emulsion is far less complete than that observed for the emulsion from the material of group 1.
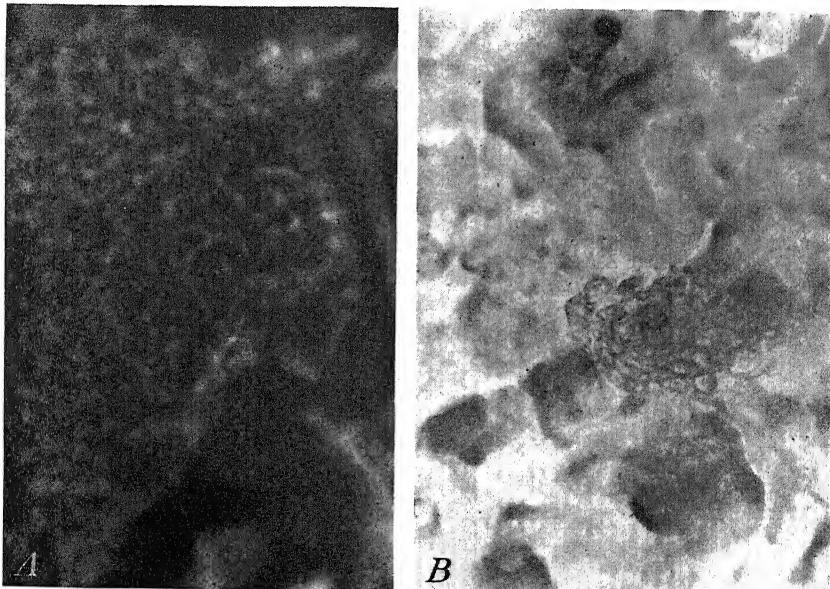
FIGURE 11.—*A*, Film of emulsion of 7.5 percent oil, 1 percent bentonite (group 1) on paraffin surface. × 1,000. *B*, Film of Summer-Mulsion (group 2) on paraffin surface. × 450.

CONCLUSIONS

This study gives evidence that films of oil emulsions possess a microscopically visible structure. Such a structure may give some information on the physical properties of the emulsion systems with respect to the degree of adherence and coverage, and to their stability; it also may reveal the distribution and the proportions of their phases.

Examination of microscopical film structures may be useful in testing oil emulsions which are intended to be applied as sprays on plant foliage. With respect to their use for reducing transpiration losses the efficiency of spray materials will depend greatly upon the permeability and adhesion of the dry film, as well as upon its extent of coverage and thickness, characteristics which can be determined partly by the patterns of their slide coatings. However, the diversity of leaf surfaces implies that a spray material may be highly effective with one species and less so with another; therefore a great variability in results is to be expected.

Observations of the behavior of spray materials on natural leaf surfaces certainly would be more informative than this study on glass

slides. However, no direct method for this purpose has been developed yet. Microscopic details of film structures on fresh leaves cannot be made visible, because the substratum on which films are applied have to be transparent in order to reveal pictures. On the other hand, treatments of leaves which might effect the desired transparency would alter the surface conditions to such an extent that any conclusions drawn from such created artifacts would be erroneous and therefore less valid than the information obtained by the method used in this study.

As to insecticidal spray materials, the contact angle and the spreading and wetting properties under existing conditions are decisive; nevertheless, the character of the configurative features of the dry film may contribute some information as to its prospective efficiency in insect control.

SUMMARY

Spray materials, which are oil-in-water emulsions, have been microscopically studied with respect to their film formation and structure on glass and other surfaces. The materials used were of two types, and were classified as group 1 and group 2.

The materials of group 1 are composed of dispersions of vegetable oils in water, with the presence of the ammonium salts of fatty acids, proteins, and bentonite, to form the system of emulsification and to give added stability. They have a high viscosity and good wetting, spreading, and quick-drying properties.

The materials of group 2 are the so-called proprietary oil emulsions. They consist of more or less refined petroleum oils, dispersed in water, and are offered in trade as insecticides. The emulsifying ingredients are not precisely specified, being trade secrets. The emulsions in this group vary in viscosity and stability. They differ from the materials of group 1 principally in the less effective character of their emulsifying and stabilizing systems.

Thinly spread layers of the emulsions of these two groups, upon receptive surfaces, dry and form adherent, solid, and more or less continuous films. Successive stages in this drying process were followed by observations with respect to the emulsions of group 1. These showed the progressive development of a fixed "honeycomb" structure, effected by aggregates of the original particles. The final distribution of phases in this honecomb structure varied with the proportional composition of the emulsion, on a given type of surface.

Successive observations on the materials of group 2 during film formation were not made. Comparison with the materials of group 1 is possible, therefore, only on the basis of the finally dried and fixed films. In general, the films of the materials of group 2 showed far less tendency, on glass surfaces, towards definite configurations, formed by aggregated particles and woven into distinctive patterns. Only under the conditions of particular ways of exposure of the surface to the spray, and the use of specially devised surfaces for the spray's reception, did formal patterns appear. Some of these were lacelike, others medallionlike, in form.

Different types of surfaces, besides glass, were prepared and used for the materials of both groups. The substratum was found to exert considerable influence on the formation of the films imposed upon it as well as on the patterns of the films.

A special case was that of a substratum of paraffin, since a paraffin film, upon solidification, develops a surface ptatern and contour of its own. Hence, besides the result as decided by the mutual reactions between surface energies of the emulsion and the substratum, there was the effect from the force of gravity which tended to localize the liquid spray in the depressions of the paraffin surface, leaving the elevated ridges uncoated. This particular effect was more pronounced with the emulsions of group 2 than with those of group 1.

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DIFFERENTIATION OF CERTAIN CRUCIFER VIRUSES BY THE USE OF TEMPERATURE AND HOST IMMUNITY REACTIONS [1]

By Glenn S. Pound, formerly *research assistant*, and J. C. Walker, *professor of plant pathology, Wisconsin Agricultural Experiment Station*

INTRODUCTION

During the several years that cabbage (*Brassica oleracea* var. *capitata* L.) mosaic has been under observation in southeastern Wisconsin, it has been noted that type and severity of symptoms vary considerably with the prevailing air temperature. Initial symptoms of the mosaic disease in the field in Wisconsin usually consist of pronounced vein clearing, vein banding, and a coarse distorting mottle. As the temperature at which the plants grow gradually increases, the vein clearing and vein banding become less intense, often being completely masked, and the mottle becomes more prevalent and pronounced. Toward the end of the season, however, as the plants mature in decreasing average temperatures, the mottle symptom gradually recedes and the vein clearing and vein banding again attain prominence. Walker, LeBeau, and Pound (*16*)[2] have recently shown that these two types of symptoms are due to two distinct viruses operating together within the host plant, although evidently affected differently by high and low temperatures. The mottle symptom is due to a strain of *turnip virus* 1 Hoggan and Johnson (*3*) known as cabbage virus A, and the vein clearing symptom is due to a strain of *cauliflower virus* 1 Tompkins referred to herein as cabbage virus B (*16*). In nature these two viruses commonly occur together in host plants, at least in Wisconsin and in the Pacific Northwest, and there is no doubt that Larson and Walker (*5*) in earlier experiments were dealing with this virus combination.

In 1937 Tompkins (*10*) described a mosaic disease of cauliflower which he stated occurred in the cool coastal valleys of California. In 1938 Tompkins, Gardner, and Thomas (*15*) described a virus disease of cabbage which they named black ring. They made no study of the temperature relations of this disease but stated that it occurred chiefly in the cool coastal valleys of California during the winter months and that it was uncommon in the summer.

Walker et al. (*16*) have shown that a very close relationship exists between cabbage virus A and the black ring virus on the one hand, and between cabbage virus B and the cauliflower mosaic virus on the other hand. This paper is a report of studies directed toward the distinction of the two virus groups and of strains within each group by differential temperature reactions and by host immunity tests.

MATERIALS AND METHODS

Cabbage viruses A and B used in this study were those employed in previous investigations in this laboratory (*16*). Cultures of the black ring and cauliflower mosaic viruses were supplied by Dr. C. M. Tompkins of the University of California. All four viruses were maintained in stock plants in aphidproof cages. Frequent inoculations were made to young cabbage plants in order to provide a constant supply of inoculum. All inoculations were made by rubbing leaves with absorbent cotton which had been dipped in juice extracted from diseased plants. Powdered carborundum was used regularly as an abrasive on all hosts. Greenhouses were fumigated weekly with vapors of nicotine and naphthalene to control insects.

When the reaction of the viruses on cabbage at various temperatures was being studied, young plants of Jersey Queen cabbage were inoculated with each virus or virus combination and kept in greenhouses with constant air temperatures of 16°, 20°, 24°, and 28° C. An equal number (usually 15) of healthy and inoculated plants was used at each temperature. Each experiment was repeated several times.

Reactions of all other hosts were determined by at least three separate tests with a minimum of five plants in each inoculation with each host, except in the reactions of the wild mustards to virus A and to the black ring virus, in which case only one trial each was made.

EXPERIMENTAL RESULTS

TEMPERATURE RELATIONS

REACTION OF CABBAGE TO THE INDIVIDUAL VIRUSES

It can be seen from table 1 that the incubation period of each of the four viruses increased in length as temperature decreased. At 16° and 20° C. the black ring virus characteristically produced symptoms 2 to 3 days earlier than virus A, but at higher temperatures both viruses produced symptoms concurrently. The incubation periods of virus B and cauliflower mosaic virus were practically identical. However, as will be pointed out later, virus B developed symptoms on certain cruciferous hosts 1 to 10 days later than the cauliflower mosaic virus.

TABLE 1.—*Effect of air temperature upon the incubation period of cabbage virus A, cabbage black ring virus, cabbage virus B, and cauliflower mosaic virus in cabbage plants in the greenhouse*

Virus	Incubation period at—			
	16° C.	20° C.	24° C.	28° C.
	Days	*Days*	*Days*	*Days*
Cabbage A	16–20	12–14	7–8	5–6
Black ring	14–18	10–12	7–8	5–6
Cabbage B	19–21	15–16	10–12	9–10
Cauliflower mosaic	18–21	14–17	10–12	9–10

A study of the symptoms produced at different temperatures revealed that the four viruses fell into two groups in regard to their reactions on cabbage at high and at low temperatures. In one group,

containing cabbage virus A and the black ring virus, mottling, stunting, distortion, and bloom reduction were very severe at 28° C. and very mild at 16°. Necrosis with the black ring virus was much more severe and pronounced at 16° and 20° than at 24° and 28°. Within this group sharp differences were noted between the reactions of virus A and the black ring virus. At 28° and 24° virus A was markedly more severe than the black ring virus, producing a severe mottle accompanied by extreme leaf distortion and severe chlorosis; the black ring virus caused much less distortion and stunting and practically no diffuse chlorosis. At 16° and 20°, on the other hand, the black ring virus was more severe than virus A, mottling and stunting were more pronounced, and necrosis was very much more common. The difference in reactions of virus A and the black ring virus at high and low temperatures is of special interest because the two viruses occur in different regions. Virus A is prevalent in the midwestern and northern States in which its economic hosts are primarily summer crops. Its season of activity is therefore largely in the summer. The black ring virus occurs in the cool coastal valleys of California where economic hosts are cultivated throughout most of the year (*10*), but the prevailing air temperatures are relatively low. Moreover, Tompkins et al. (*15*) state that epidemics of the black ring disease occur chiefly in the winter and are uncommon in the summer. Although the two viruses are very similar, it may be that they have become established in their respective regions because the prevailing air temperatures in these regions are favorable for their activity.

In the other group, containing cabbage virus B and the cauliflower mosaic virus, intensity of symptom development increased with decrease in temperature. At all temperatures symptoms appeared as a chlorotic vein clearing which was prominently expressed but which rapidly became completely masked at 24° and 28° C. At 16° and 20° vein clearing and chlorotic vein banding were prominent and persistent. At these temperatures enations occurred as warty, translucent excrescences along the veins on the undersurface of the leaf more commonly than at higher temperatures. The enations, although sporadic in their development, appeared to be associated with a pronounced expression of vein clearing and vein banding. Hence they were more common at low temperatures and occurred with equal frequency with virus B and the cauliflower mosaic virus. Stunting at low temperatures, in spite of pronounced symptoms, was no greater than at higher temperatures and was less than that caused by virus A and the black ring virus. The effect of temperature upon disease development for the cauliflower mosaic virus was exactly parallel to that for virus B. The two viruses differed in that symptoms of the cauliflower mosaic virus were always milder than those of virus B. The fact that B-infected plants were more chlorotic than those infected with the cauliflower mosaic virus often served as a sharp point of distinction between the two viruses.

REACTION OF CABBAGE TO VARIOUS COMBINATIONS OF THE VIRUSES

When viruses A and B were inoculated simultaneously into the same plants, the degree of interaction was somewhat clearly defined by the temperature effect on symptom expression of the virus combination. An examination of figure 1 will show that the combined
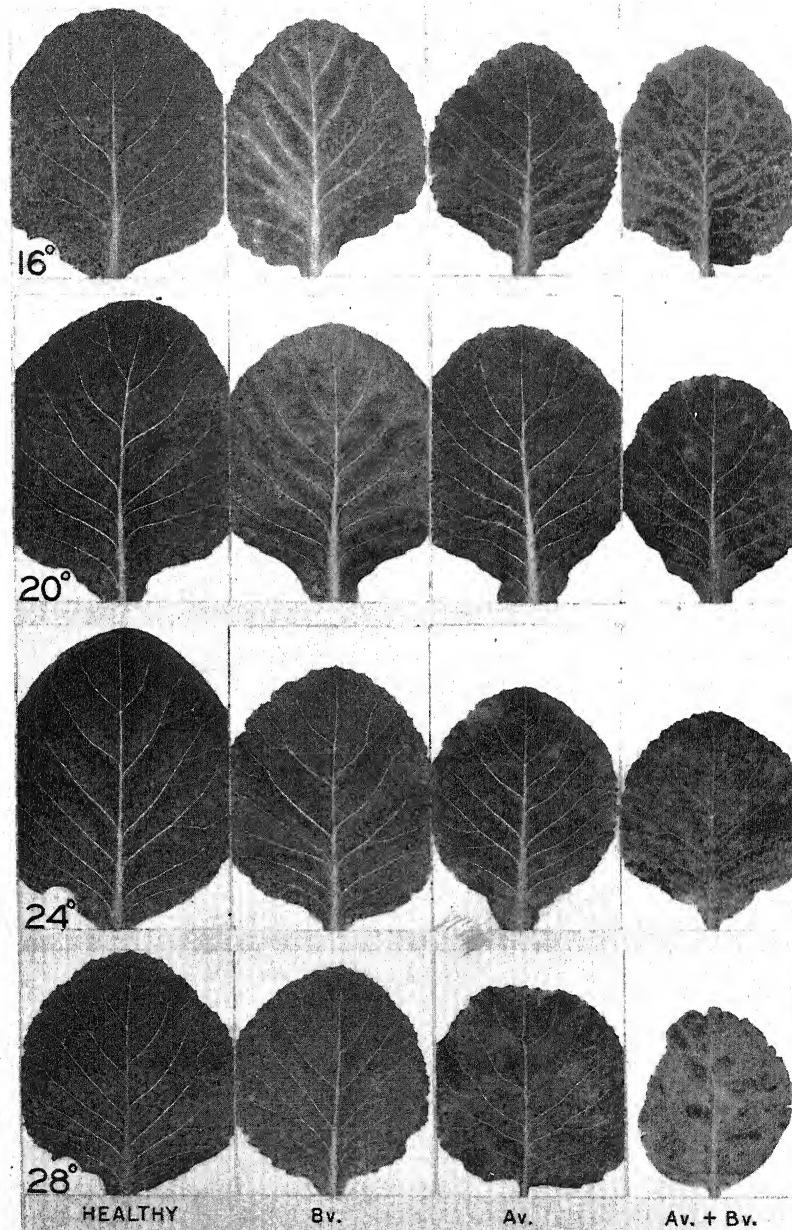
FIGURE 1.—Systemic symptoms on young leaves of Jersey Queen cabbage produced by cabbage virus B (Bv.), cabbage virus A (Av.), and cabbage viruses A and B (Av.+Bv.) together at various temperatures. Note that symptoms of virus B are masked at 28° and 24° C. but are prominently expressed at 20° and 16°C. Note also the progressive increase in severity of symptoms of virus A with increase in temperature and the pronounced increased severity at 28° and 24° when viruses A and B occur together.

effect of the two viruses was more severe than that of either virus alone. At higher temperatures the addition of the B component to virus A resulted in the appearance of vein clearing and rapidly changed the coarse chlorotic mottle into a fine mottle with much more chlorosis and increased severity, with the result that ultimate symptoms bore little resemblance to those of either virus alone. Stunting, leaf distortion, bloom reduction, and premature leaf abscission were very severe. Necrosis, quite uncommon to virus A, became very severe on some plants. Symptoms (other than stunting and chlorosis) of virus B alone at 24° and 28° rapidly became masked, but the presence of this virus in combination with virus A at these temperatures effected a striking change in the type and severity of symptoms produced. Whether the presence of this virus enhanced the activity of virus A, or vice versa, or whether this symptom change was the additive result of the effect of each individual virus on the metabolism of the host is a question prompting further investigation. At 16° and 20° the combined effect of viruses A and B resulted in more stunting and chlorosis than for either virus alone but there was no change in type of symptoms as at 28°. The effect of each virus was evident, and there was little apparent interaction between them. The activity of virus A was reduced such that little additive effect was evident, and the ultimate symptoms were largely those of virus B.

A few days after plants showing severe A+B symptoms were moved from a house at 28° to one at 16° C., subsequently developing leaves showed only a very mild mottle and a vein clearing which gradually increased in prominence. Symptoms on young leaves showed little resemblance to those on plants kept at 28°. Likewise, a few days after A+B plants showing typical low-temperature symptoms were transferred from 16° to 28°, the conspicuous vein clearing and vein banding disappeared, and young leaves showed a severe fine mottle accompanied by much stunting and distortion. When these two groups of plants were again removed to the houses of their original incubation, they once again developed symptoms characteristic of that specific temperature. These reversible temperature reactions of plants infected with both viruses fully confirm observations made of the disease in the field.

When plants showing systemic symptoms of virus A were reinoculated with virus B, or vice versa, there developed at each temperature symptoms typical of the A+B combination after the respective incubation period of the second virus introduced. Furthermore, the two viruses were easily recovered individually from such plants by the use of heat inactivation and differential hosts as described by Walker et al. (*16*). The fact that there was no immunization of the host by virus A toward virus B and vice versa would indicate no close relationship between the two viruses.

Symptoms produced by the mixture of cabbage A and cauliflower mosaic viruses (fig. 2) were practically identical with those described for the A+B combination. Just as symptoms of the cauliflower mosaic virus alone on cabbage were slightly milder than those of virus B, the symptoms produced by this virus in combination with virus A were slightly milder than those of the corresponding A+B combination.

Combinations of the black ring virus with virus B and with the cauliflower mosaic virus were very similar to the corresponding com-
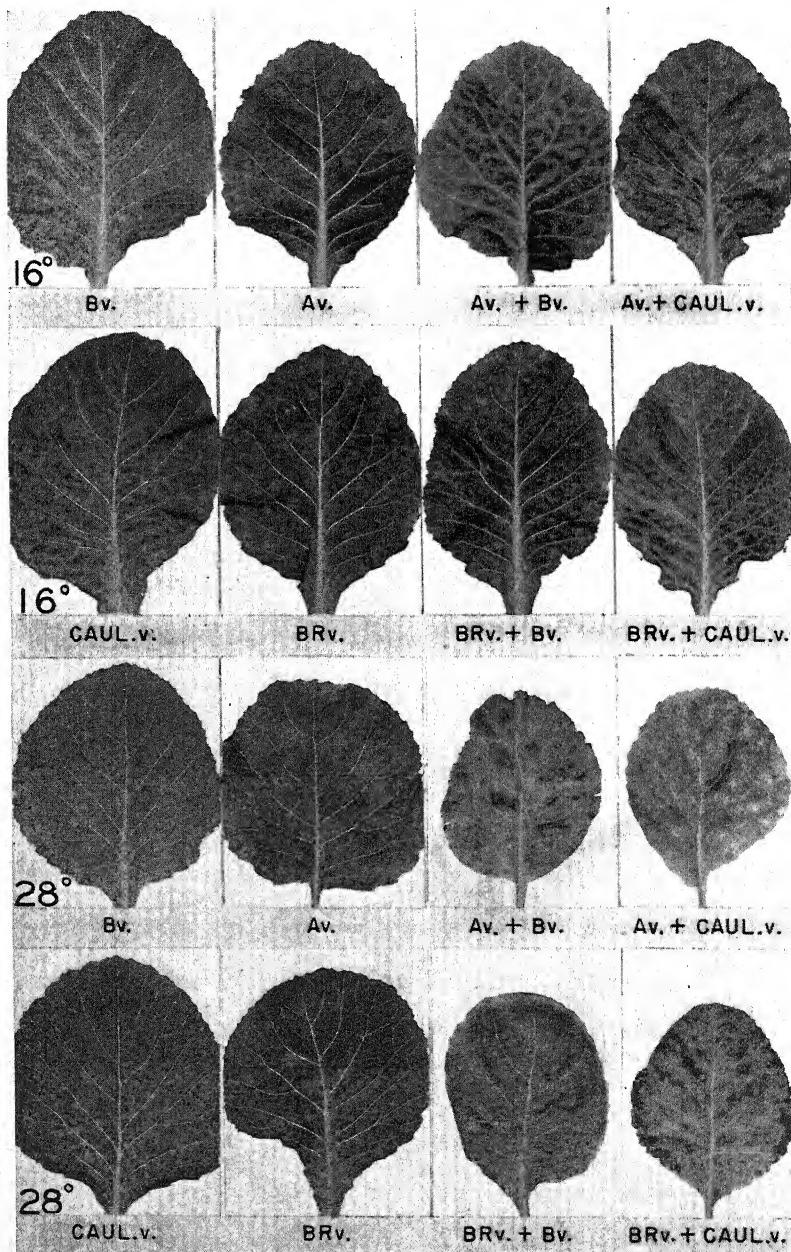
FIGURE 2.—Symptoms produced on young leaves of Jersey Queen cabbage by cabbage A (Av.), cabbage B (Bv.), cabbage black ring (BRv.), and cauliflower mosaic (Caul. v.) viruses alone and in various combinations at 16° and 28° C. Note the similarity in symptoms of virus A and the black ring virus both when alone and in combination with virus B or the cauliflower mosaic virus. Note also the similarity of symptoms of virus B and the cauliflower mosaic virus.

binations of virus A except that the differences in the temperature reactions of virus A and the black ring virus were also manifested in the combinations of these viruses. At 24° and 28° the black ring virus combinations produced less chlorosis and less stunting than did the corresponding combinations of virus A. In addition, dark-green rings and ring spots typical of the black ring virus were manifested. At 16° and 20° all combinations of the black ring virus produced more necrosis and a slightly more prominent mottle than those of virus A.

Thus in the various combinations involving viruses of the two groups the reactions of the cauliflower mosaic virus were practically identical with those of virus B, and the reactions of the black ring virus were very similar to those of virus A (fig. 2). These similarities were expected since the same relations were manifested in the reactions of each of the viruses alone. Tompkins (*10*) and Tompkins et al. (*15*) made no mention of the black ring and cauliflower mosaic viruses occurring together within the same plant in nature. Neither did they describe symptoms typical of the combined action of the two viruses.

REACTION OF SELECTED HOSTS AT VARIOUS TEMPERATURES TO CABBAGE VIRUS A AND THE BLACK RING VIRUS

When cabbage virus A and the black ring virus were studied over an extensive host range (*16*) certain hosts were noted on which the two viruses acted somewhat differently. These were studied further at 16°, 20°, 24°, and 28° C. to determine which could be used for cross-immunity tests at one or another temperature.

Nicotiana glutinosa L.

The reactions of the two viruses on *N. glutinosa* differed only in minor respects, but the effect of temperature upon symptom expression was most striking. With both viruses the effect upon the host plant was most severe at 16° and 20° and least so at 28°, a temperature reaction quite the reverse of that noted for cabbage. Moreover, the type of symptom changed with increase in temperature. At 16° primary symptoms were necrotic flecks with chlorotic halos, while systemic symptoms were vein clearing, mottle, stunting, and necrosis. At 20° the symptoms were of the same general type but more severe. At 16° and 20° black ring symptoms were slightly more severe than those of virus A. At 24° there was a shift to more mottle, less necrosis and less stunting. At 28° primary symptoms were small necrotic flecks around which conspicuous zonate rings slowly developed. Similar ring spot symptoms occurred systemically (fig. 3.) The youngest leaves showed no symptoms (even after 90 days' incubation), and it was only when leaves were separated from the growing point by four to six internodes that symptoms appeared. Repeated attempts to recover the viruses from the symptomless young leaves were unsuccessful, even after 50 days' incubation. Recovery tests from leaves showing symptoms yielded the viruses in fair concentration. No necrosis, mottling, or distortion occurred at this temperature.

Thus on this host completely different types of symptoms were produced at different temperatures, the mildest symptoms occurring at 28° C. Since *Nicotiana glutinosa* was favored by relatively high
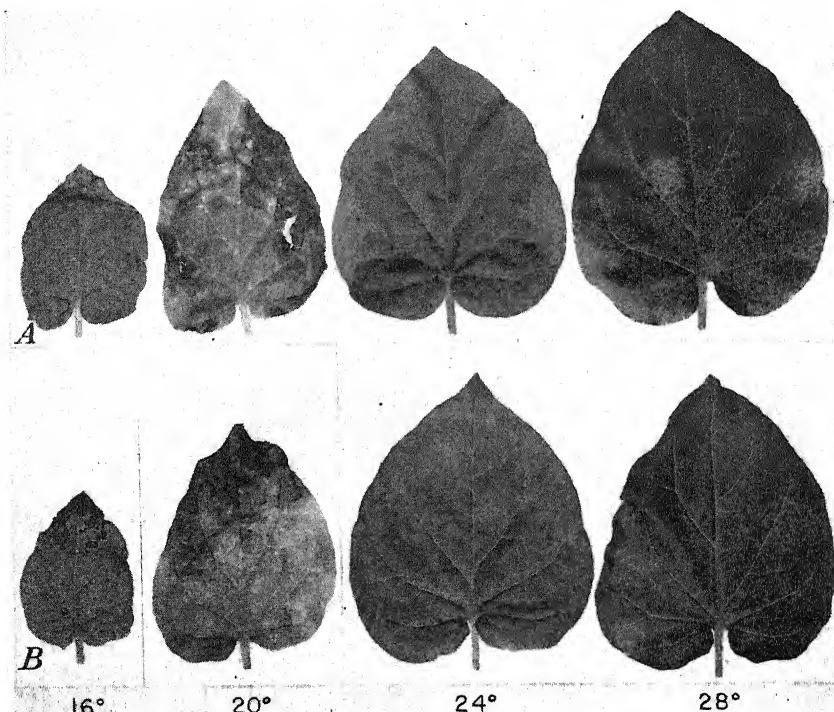
FIGURE 3.—Systemic symptoms produced by (*A*), virus A, and (*B*), black ring virus, on *Nicotiana glutinosa* at temperatures of 16°, 20°, 24°, and 28° C., respectively. All leaves are of the same age. Note the extreme similarity in the reactions of the two viruses and the effect of temperature upon symptom "type" as well as decrease of severity with increase in temperature. Compare reaction of this host to virus A with the reaction of cabbage to the same virus (fig. 1), where the disease increases in severity with increase in temperature.

temperatures, and since each virus produced its greatest effects at temperatures unfavorable to the host and its least effects at temperatures favorable to the host, it would seem that the temperature effect upon the host-virus relationship was largely that of the temperature effect upon the host. This condition has its counterpart in the effect of temperature on the reaction of cabbage to the A and black ring viruses. Cabbage makes much better growth at 16° and 20° than at 28° C. It will be remembered that the symptom severity of the two viruses on cabbage decreased with decrease in temperature except for the increase in necrosis with the black ring virus at 20°. It is often stated that plants in a vigorous growing condition develop more severe symptoms than plants in a less active state. If this be true, then the reaction of *N. glutinosa* is exceptional, both in the mild symptoms produced and in the slowness with which the two viruses invaded actively growing young leaves at 28°.

It is evident that there must be also a temperature effect upon the activity of the viruses themselves. It was pointed out earlier that both viruses show incubation periods on cabbage, a low temperature plant, which increase in length with decrease in temperature. On tobacco, a high temperature plant, the same viruses also show incu-

bation periods which increase in length with decrease in temperature. However, as will later be shown, symptoms on *Nicotiana rustica* appear concurrently at low and high temperatures. Furthermore, on many hosts virus A and the black ring virus do not show the same temperature reaction in regard to the length of the incubation period. Walker et al. (*16*) pointed out that the black ring virus produces more necrosis than does virus A. This production of necrosis, especially on cruciferous plants, is restricted almost entirely to temperatures below 24° C. On many hosts this virus is more severe at 20° than at 28°, whereas virus A almost without exception is most severe at 28°. These facts clearly indicate that, as regards temperature effects, the reaction on any host is due to the temperature effect upon the specific host-virus combination and maybe due to the temperature effect upon the host, the virus, or both. The last of these three possibilities is probably the one which usually prevails.

Nicotiana rustica L.

When *Nicotiana rustica* was used the symptoms at all four temperatures appeared almost simultaneously. At 24° and 28° differences between the two viruses were slight. The black ring virus produced primary circular chlorotic lesions at 24° and 28°; virus A produced them indistinctly and only at 24°. Both produced zonate chlorotic rings and solid chlorotic spots systemically. At 16° and 20° the reactions of the two viruses were markedly different. The black ring virus produced numerous necrotic primary lesions and numerous chlorotic systemic lesions, 4 to 5 mm. in diameter and bordered by necrotic dots; stunting and leaf distortion were marked. The primary lesions caused by virus A were largely scattered chlorotic lesions while the systemic lesions were larger, fewer in number, and stunting was less pronounced. The systemic lesions of the two viruses are compared in figure 4.

Nicotiana multivalvis Pursh

On *Nicotiana multivalvis* the differences were most pronounced at 16° C., where virus A produced no primary symptoms while the black ring virus produced a conspicuous pattern of small white rings and ring spots. Systemically the symptoms were most severe at 16°, decreasing with increase in temperature as with *N. glutinosa* but without any marked change in symptom type. The black ring virus was the more severe at 16°, the difference becoming less pronounced with increase in temperature.

Wild Mustards

The effects of the two viruses on young plants of *Brassica juncea* (L.) Coss. (Indian mustard), *B. nigra* (L.) Koch (black mustard), *B. arvensis* (L.) Ktze. (charlock), and *B. campestris* L. (wild yellow mustard) at 20° were easily distinguishable (fig. 5). The black ring virus produced numerous, angular necrotic lesions on inoculated leaves. Necrosis spread, became systemic, and plants died within 3 weeks after inoculation. Except on charlock, virus A produced no primary necrotic lesions, and plants were not killed until several days
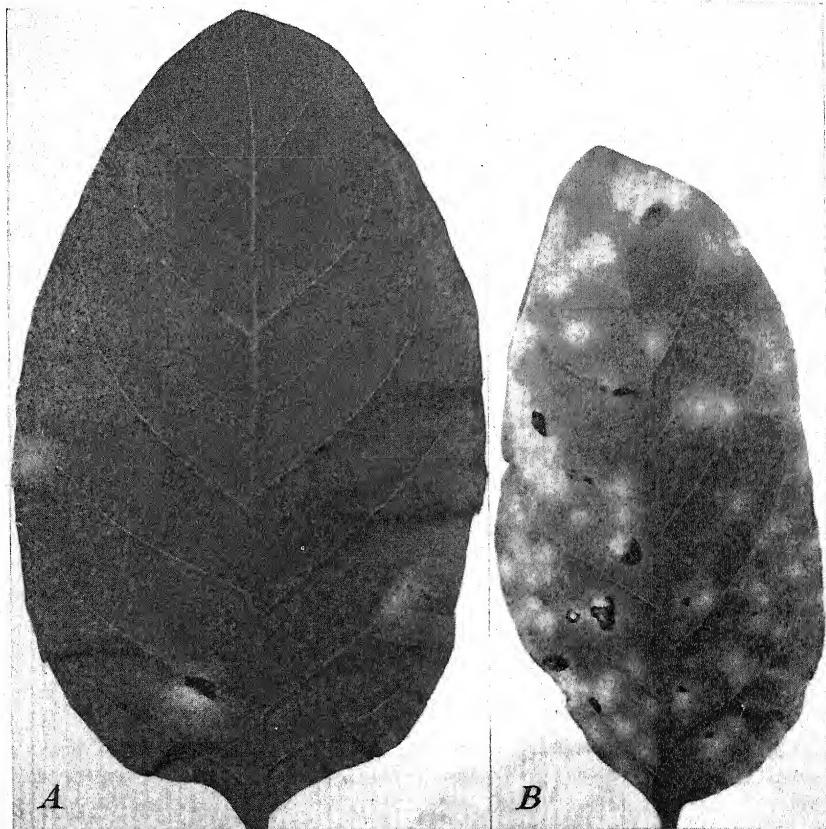
FIGURE 4.—Systemic symptoms produced on *Nicotiana rustica* at 16° C.: *A*, Cabbage virus A; *B*, Black ring virus. Note the more pronounced mottle and more severe stunting caused by the black ring virus.

after death occurred to black-ring-infected plants. Symptoms were markedly milder than those produced by the black ring virus. At 28° disease development was so rapid with both viruses that plants were killed within 5 to 8 days after inoculation.

Brassica oleracea var. *gemmifera* DC. (Brussels sprouts)

At 16° and 20° C. symptoms of the two viruses on Brussels sprouts (var. Long Island Mammoth) were markedly different. The black ring virus produced a severe necrosis on inoculated leaves as circular lesions which expanded and coalesced, causing the entire leaf to die. Systemic symptoms appeared as circular chlorotic rings and spots which rapidly became necrotic to produce a conspicuous pattern of black rings and streaking of the veins. Virus A produced only chlorotic lesions, or no symptoms at all, on inoculated leaves and a systemic mottle which was very mild and never became necrotic until 4 to 5 weeks after inoculation. At 20° the effects of the two viruses were so different that they might easily have been considered as two distinct diseases (fig. 6). At 28° the symptoms of the two viruses were indistinguishable.
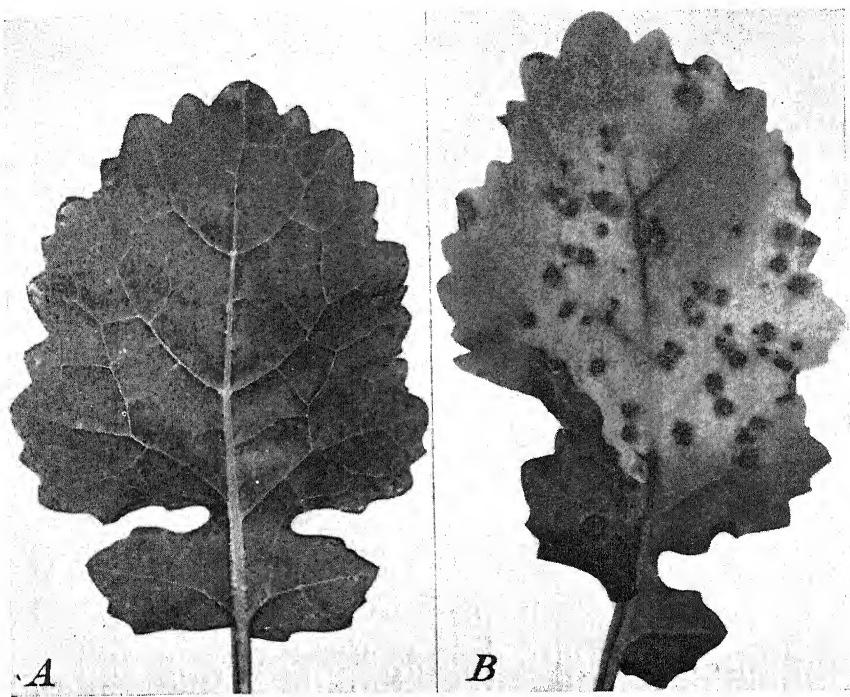
FIGURE 5.—Symptoms produced on inoculated leaves of Indian mustard (*B. juncea*) at 20° C.: *A*, Virus A; *B*, black ring virus.   Note the extreme difference in the effects produced by the two viruses.
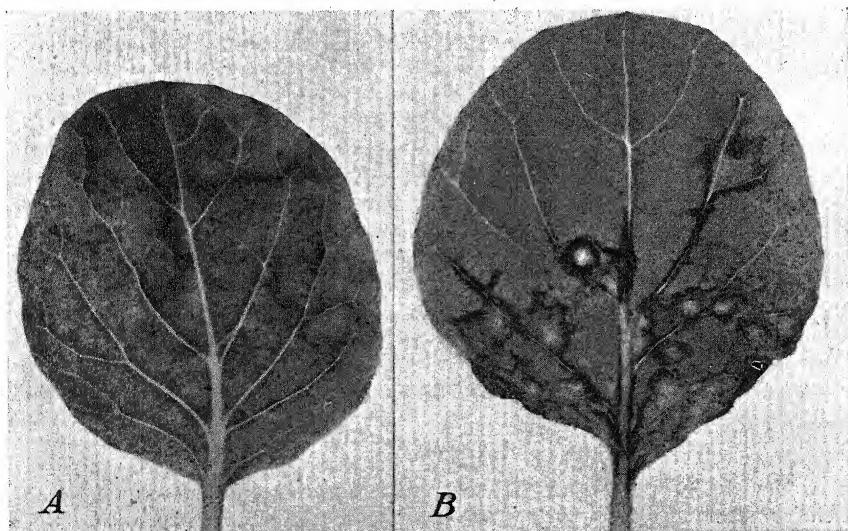
FIGURE 6.—Systemic symptoms produced on Brussels sprouts at 20° C.: *A*, Mild mottle caused by virus A; *B*, Severe necrotic pattern produced by the black ring virus.

REACTION OF VARIOUS HOSTS TO CABBAGE VIRUS B AND CAULIFLOWER MOSAIC VIRUS

Brassica pekinensis (Lour.) Rupr. (Chinese cabbage)

On Chinese cabbage (var. Chihli) at 16° C. the cauliflower mosaic virus produced symptoms after 18 to 21 days as a conspicuous chlorotic vein clearing. This symptom became progressively more pronounced, and leaf. midribs developed a marked curvature. Leaf laminae became severely ruffled and stunted, resulting in a conspicuous rosette in which a few old leaves showed no malformation, while severely stunted and distorted young leaves formed the central cluster. Virus B produced symptoms some 7 to 10 days later than the cauliflower mosaic virus. These appeared as vein clearing and leaf curvature and were not unlike the symptoms caused by the cauliflower mosaic virus except that severe stunting and rosetting did not occur. These much milder symptoms served as a sharp point of distinction between the two viruses. At 20° and 24° the symptoms of the two viruses became increasingly more alike and at 28° they were indistinguishable. In general, rosetting and vein clearing decreased with increase in temperature while chlorosis and stunting increased. As a means of differentiating the two viruses this host served best at 16°.

Wild Mustards and Rape

The reactions of the two viruses at 20° and 28° on young plants of *Brassica arvensis* (charlock), *B. campestris* (wild yellow mustard), *B. juncea* (Indian mustard), *B. hirta* Moench, (*B. alba* (L.) Rabenh. (white mustard)) and *B. napus* L. (rape var. Dwarf Essex) were studied.

At 20° symptoms of the cauliflower mosaic virus became apparent in 12 to 15 days as a conspicuous chlorotic vein clearing and mild mottle. Young leaves exhibited a marked curvature of the midrib and a severe ruffling and stunting of the lamina. These severely stunted and inrolled young leaves produced a marked rosette on all hosts. Many plants of the wild mustard hosts were ultimately killed. Symptoms of virus B appeared 2 to 4 days later than those of the cauliflower mosaic virus as vein clearing followed by mild mottle with curving and wrinkling of the leaves. No marked rosette of young leaves developed on any of the hosts, and symptoms were much milder than those caused by the cauliflower mosaic virus.

At 28° the cauliflower mosaic virus again produced symptoms earlier and much more severe than did virus B. Stunting, distortion, mottling, and chlorosis were more severe, but rosetting was less pronounced than at 20°. Symptoms were very severe and most plants were finally killed. At both 28° and 20° the two viruses were easily separable by the degree of symptom severity rather than by the type of symptom.

HOST IMMUNITY REACTIONS IN CABBAGE

In the preceding section differential temperature reactions between virus A and the black ring virus and between virus B and the cauliflower mosaic virus on several hosts were described. As a result of these critical studies of the effects of temperature on symptom expression, certain host reactions were found which differentiated between the viruses in question so distinctly that they were selected

for use in cross-immunity tests.   The efficacy of these selected hosts in differentiating between the viruses in question depended upon the use of a temperature which insured the expression of the differentiating symptoms.   Although the differentiation was more distinct at one specific temperature, the hosts used were tested at all four temperatures to widen the scope of the test.

SPECIFIC IMMUNITY AGAINST THE BLACK RING VIRUS PRODUCED BY VIRUS A

Immunological tests involving virus A and the black ring virus were set up as follows: Each virus was inoculated to 10 cabbage plants.   After all plants developed systemic symptoms the A-infected plants were reinoculated with the black ring virus, and the plants infected with the black ring virus were reinoculated with virus A, inoculations being made on systemically infected leaves. At the same time 10 healthy plants of the same age were inoculated (to serve as controls) with each of virus A, the black ring virus, and a mixture of the 2 viruses.   Two weeks after systemic symptoms appeared in the 3 groups of control plants inoculations were made from all of the 5 groups of 10 plants each to *Nicotiana multivalvis*, *N. rustica*, and *Brassica oleracea* var. *gemmifera* at temperatures of 16°, 20°, 24°, and 28°.   Extracts were taken from young leaves above inoculated leaves.

Symptoms produced on plants inoculated with extracts taken from the A-infected plants reinoculated with the black ring virus agreed in every respect with symptoms produced on these hosts by virus A alone.   On all hosts at all temperatures symptoms showed no resemblance to those of the black ring virus but, on the contrary, were exactly identical with those of virus A as described in the preceding section.   Correspondingly, extract from cabbage plants infected with the black ring virus and reinoculated with virus A produced symptoms exactly like those of the black ring virus alone.   This, of course, was expected since the more severe symptoms of the black ring virus would cover up the mild symptoms of virus A if virus A were present. Figures 7 and 8 show the results of the inoculations to Brussells sprouts and *N. rustica*, respectively.

Inoculum taken from plants infected with the A-black-ring mixture produced symptoms similar to those of the black ring virus alone. This indicated that virus A does not inhibit the establishment of the black ring virus when the two are introduced into the plant simultaneously.

In another test 40 young Brussels sprouts plants were inoculated with virus A and incubated at 20°.   After plants developed systemic symptoms, 20 of the infected plants were reinoculated with the black ring virus.   At the same time 20 healthy plants of the same age were inoculated with the black ring virus.   After 10 days the healthy plants inoculated with the black ring virus had developed a severe necrosis on inoculated leaves, and subsequent systemic necrosis was severe and typical of the black ring virus.   Plants which were infected with virus A and reinoculated with the black ring virus developed no necrosis on inoculated leaves and no systemic necrosis until several days after systemic necrosis appeared in the black ring control plants.

In still another test 10 plants of *Nicotiana multivalvis*, infected with
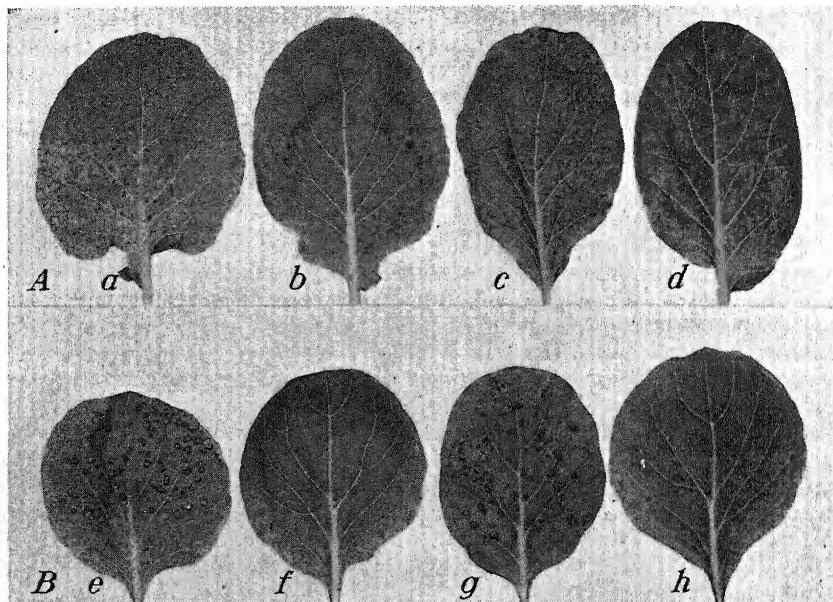
FIGURE 7.—*A*, Systemic symptoms produced on Brussels sprouts at 16° C. when inoculated with extracts taken from cabbage plants used in the cross-immunity tests of virus A and the black ring virus. *A*, Leaves from plants inoculated with: *a*, Extract taken from cabbage infected with black ring virus alone; *b*, extract from cabbage infected with virus A alone; *c*, extract from cabbage infected with black ring virus and reinoculated with virus A; *d*, extract from cabbage infected with virus A and reinoculated with the black ring virus, respectively. *B*, primary symptoms produced on inoculated leaves (*e, f, g, h*) of same plants.

virus A and showing only a mild systemic mottle, were inoculated with the black ring virus. At the same time an equal number of healthy plants of the same age were inoculated with the black ring virus. After 5 days at 24° the healthy plants inoculated with the black ring virus developed numerous white rings and ring spots on inoculated leaves. Plants infected with virus A and reinoculated with the black ring virus failed to develop primary symptoms.

These tests would indicate a strain relationship between virus A and the black ring virus if the failure of the black ring virus to establish itself in plants infected with virus A were due to a specific acquired immunity.

SPECIFIC IMMUNITY AGAINST VIRUS A PRODUCED BY THE BLACK RING VIRUS

In an extensive host range study of virus A and the black ring virus (*16*) one host was found which appeared to be completely differential for the two viruses. This host, *Solanum integrifolium* Poir. (Chinese scarlet eggplant), was found to develop conspicuous, necrotic, primary lesions when inoculated with virus A at 28°, 24°, and 20°. This necrosis became systemic and appeared as numerous lesions 2 to 4 mm. in diameter and as streaking of the veins on young leaves. Leaf abscission resulting from the necrosis was marked. No mottle symptom developed. In four separate trials the black ring
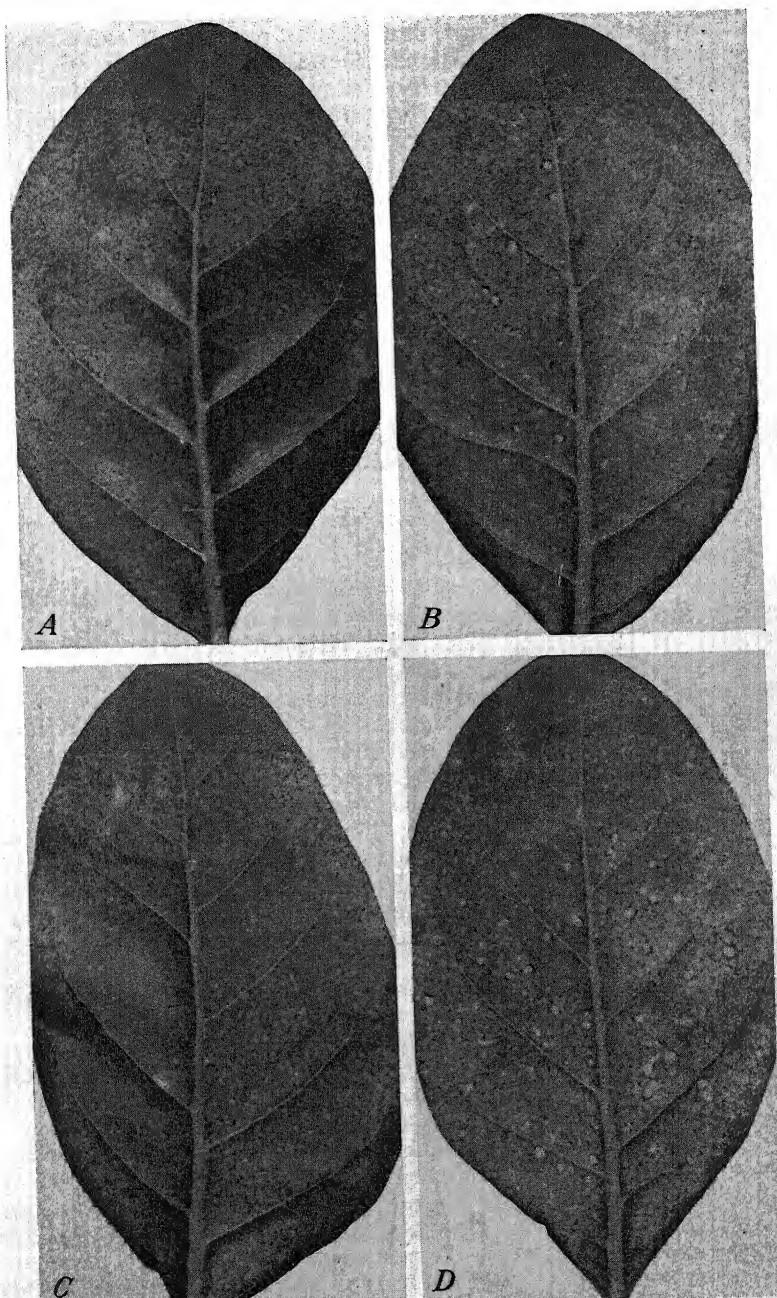
FIGURE 8.—Symptoms produced on inoculated leaves of *Nicotiana rustica* at 16° C., with extracts taken from cabbage plants used in cross-immunity tests of virus A and the black ring virus.   Leaves taken from plants inoculated with: *A*, Extract taken from cabbage infected with virus A alone; *B*, extract from cabbage infected with black ring virus alone; *C*, extract from cabbage infected with virus A and reinoculated with the black ring virus; *D*, extract from cabbage infected with black ring virus and reinoculated with virus A.

virus produced no symptoms, and attempts to recover the virus from the symptomless plants were unsuccessful.

No symptoms were produced on 27 plants of *Solanum integrifolium* with virus taken from cabbage plants infected with the black ring virus and reinoculated with virus A. Neither did extracts from the plants infected with the black ring virus alone produce symptoms. Virus taken from cabbage plants infected with virus A and reinoculated with the black ring virus produced symptoms similar to those of virus A alone (fig. 9). Inoculum taken from cabbage plants infected with the A-black-ring mixture produced symptoms similar to those produced by virus A, indicating that the black ring virus does not inhibit the establishment of virus A in plants when the two viruses are introduced into the plants simultaneously. Thus in this test (fig. 9, *C*) the black ring virus did not prevent the establishment of virus A in systemically infected cabbage plants. However, when cabbage plants were first inoculated with black ring virus and then reinoculated with virus A the latter produced no symptoms (fig. 9, *D*).

SPECIFIC IMMUNITY AGAINST CAULIFLOWER MOSAIC VIRUS PRODUCED BY VIRUS B

To test the ability of virus B to protect cabbage plants against infection with the cauliflower mosaic virus a test similar to that just described for the A and black ring viruses was used. Ten young cabbage plants were inoculated with each of virus B and the cauliflower mosaic virus. After systemic symptoms appeared in all plants the B-infected plants were reinoculated with the cauliflower mosaic virus, and the plants infected with the cauliflower mosaic virus were reinoculated with virus B. At the same time virus B, cauliflower mosaic virus, and a mixture of the 2 viruses were each inoculated to 10 healthy plants of the same age. Two weeks after systemic symptoms appeared in the 3 groups of control plants, inoculations were made from each of the 5 groups of 10 plants to Chinese cabbage plants at 16°, 20°, 24°, and 28° C., a minimum of 5 test plants being used in each inoculation. The experiment was repeated 3 times and gave the same results each time.

Symptoms produced by an extract taken from B-infected plants which were reinoculated with cauliflower mosaic virus were identical at each temperature with those produced by virus taken from plants infected with virus B alone. Symptoms produced by extracts taken from plants infected with the cauliflower mosaic virus alone and from plants infected with the cauliflower mosaic virus but later reinoculated with virus B were identical. Inoculum taken from cabbage plants infected with a mixture of the 2 viruses produced symptoms characteristic of the cauliflower mosaic virus. In figure 10 are shown the results obtained in one of the experiments. One month later a new series of inoculations was made from the same groups of cabbage plants to young Chinese cabbage plants. Results similar to those in the first inoculations were obtained. These tests show that cabbage plants systemically infected with virus B are protected by the virus against infection by the cauliflower mosaic virus and indicate a strain relationship between virus B and the cauliflower mosaic virus.
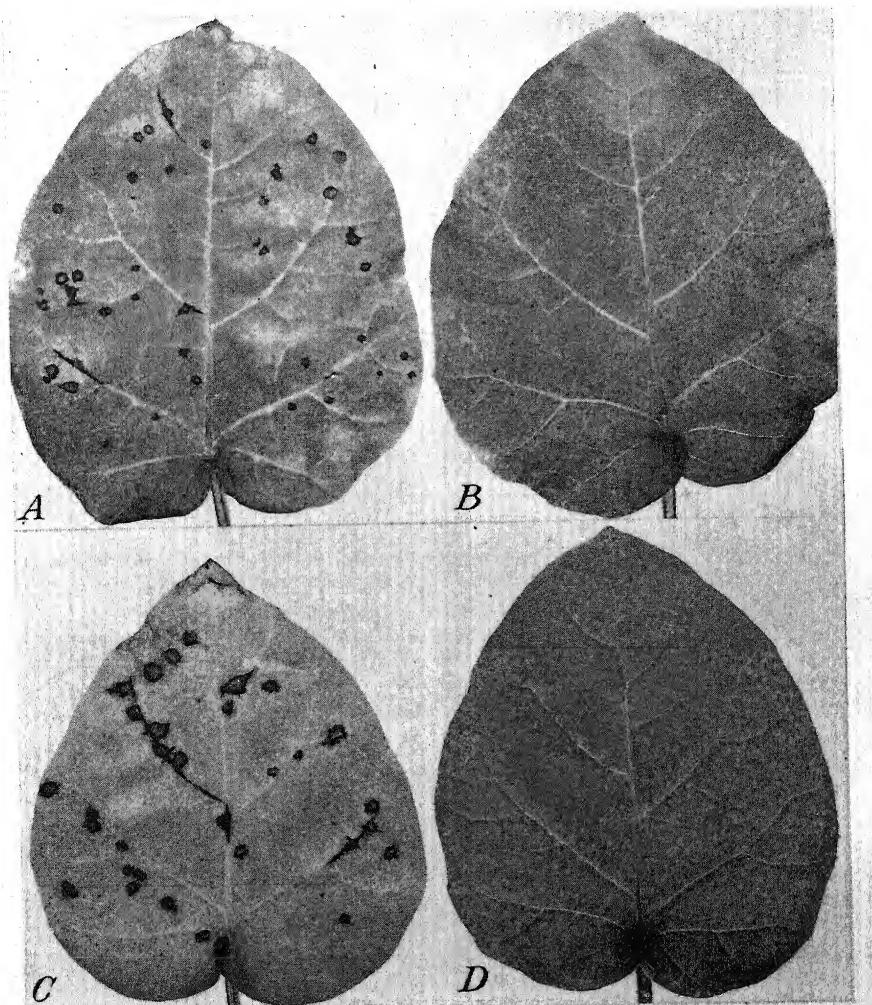
FIGURE 9.—Symptoms produced on inoculated leaves of *Solanum integrifolium* with extracts taken from cabbage plants used in the cross-immunity tests of the A and black ring viruses. Leaves from plants inoculated with: *A*, Extract taken from cabbage infected with virus A alone; *B*, extract from cabbage infected with black ring virus alone; *C*, extract from cabbage infected with virus A and reinoculated with the black ring virus; *D*, extract from cabbage infected with black ring virus and reinoculated with virus A. Note that the black ring virus produced no symptoms.
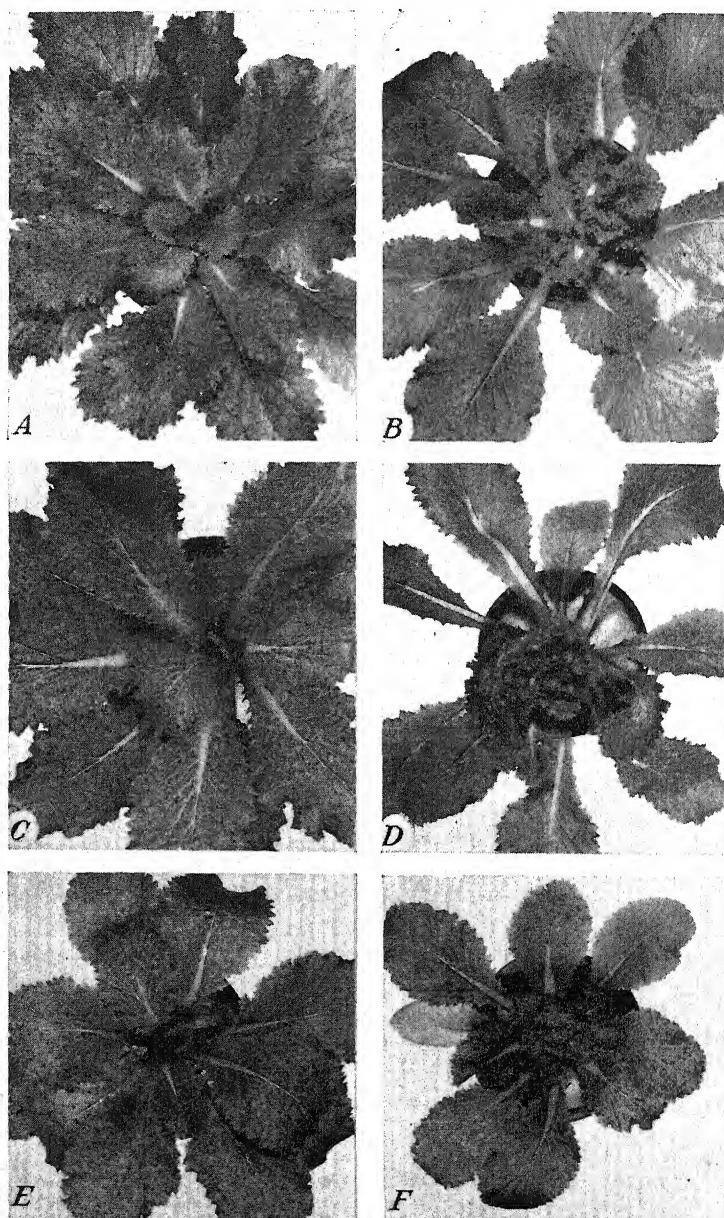
FIGURE 10.—Symptoms produced on Chinese cabbage at 20° C. 30 days after inoculation with extracts taken from cabbage plants used in cross-immunity tests of virus B and the cauliflower mosaic virus. *A*, Extract taken from cabbage infected with virus B alone; *B*, extract from cabbage infected with the cauliflower mosaic virus alone; *C*, extract from cabbage infected with virus B and reinoculated with cauliflower mosaic virus; *D*, extract from cabbage infected with cauliflower mosaic virus and reinoculated with virus B; *E*, uninoculated control; *F*, extract from cabbage inoculated with a mixture of virus B and cauliflower mosaic virus. Note that extract taken from plants infected with virus B reinoculated with cauliflower mosaic virus produces symptoms indistinguishable from those of virus B alone.

DISCUSSION

The effect of temperature upon host-virus relationships has received disproportionately little attention in comparison with other phases of virus investigations. Many comparisons of viruses today are based upon symptoms and properties of the viruses. Evidence presented in this paper shows that type and intensity of symptoms may depend greatly upon the temperature to which the host plants are exposed. Other experiments under way indicate that temperature may radically alter the activity and concentration of the virus within the host. Since the determination of the physical properties of the viruses is influenced by the concentration of the virus in the expressed sap, it is suggested that the temperature under which they develop in the host may indirectly affect the physical properties of the virus as ordinarily determined. Thus in making comparison in symptomatology and properties of viruses prime consideration should be given to the temperatures of the environments in which the symptoms develop and the viruses multiply. High temperature is often reported to reduce the severity of a given virus. Observations reported herein indicate that the effect of temperature upon disease severity depends upon the specific host-virus complex and may be due to the temperature reaction of the host, virus, or both.

The effect of temperature upon host-virus reaction may have considerable bearing on virus classification and nomenclature. The inadequacies of a system of classification based primarily upon symptomatology are revealed by the reactions of cabbage viruses A and black ring on *Nicotiana glutinosa* and other hosts at different temperatures. For instance, the symptoms of these viruses on *N. glutinosa* at 28° C. include those given by Holmes (*4*) for his family, Annulaceae, while the symptoms produced on this host at 16° are typical of those given for the family Marmoraceae. The name black ring is very appropriate for the reactions of the black ring virus on crucifers at low temperatures but very inappropriate for symptoms at high temperatures.

Many investigators have tested numerous host plants with certain viruses in the hope of finding hosts which would give a differential reaction to related viruses or a local-lesion reaction which could be used in quantitative measurement of the viruses. Results given in this report show that by testing related viruses on selected hosts at various temperatures differential reactions may be obtained which may be more marked than those obtained by testing the viruses on a large number of hosts at only one temperature. Likewise, temperature studies may provide a local-lesion reaction which could not be found by trying several hosts at only one temperature. These values of temperature studies are well illustrated by the reaction of *Nicotiana rustica* to the cabbage A and the black ring viruses. At high temperatures the two viruses were not easily distinguishable on this host, but at low temperatures their reactions were very different. Furthermore, the reactions of the black ring virus at 16° and 20° were such that they could be easily applied to quantitative studies of the virus itself. Many hosts were found in this and other investigations (*16*) that gave a local-lesion reaction to this virus at low temperatures but not at high temperatures. Most of the differentiating symptoms used

in the immunity reactions reported in this paper resulted from finding differential temperature reactions of the viruses in question.

The method of measuring specific acquired immunity employed in this investigation proved just as effective as the ordinary local-lesion-mottle test although it involved more work. Its effectiveness shows that where two related viruses have a differential reaction on any host their ability to immunize plants against each other can be tested in any plant in which they develop systemically.

Significant features in the properties and host range of various crucifer viruses are presented in table 2. These comparisons are made in order to focus attention upon possible relationships in this heterogeneous group of viruses. Although the writers are of the opinion that these viruses fall into two distinct groups, it is realized that any such relationships can be truly ascertained only by parallel studies in the same laboratory. However, the viruses do show enough common characteristics to justify a limited amount of speculation regarding their relationships.

A study of table 2 reveals that in properties the first 14 viruses listed fall more or less into 1 group, the properties of which are limited by the following values: Aging in vitro, 48–384 hours; dilution, 1–600 to 1–100,000; thermal inactivation, 50°–68° C. The greatest variation in these properties lies in the measure of dilution tolerance. The fact that different test plants were used might account for some of this variation. It is the writers' experience that, with cabbage virus A, infection on tobacco can be obtained at a lower concentration than on cabbage. This may be due to chance since much more leaf area is covered in inoculating 10 tobacco plants than in inoculating 10 cabbage plants. Furthermore, in only 1 trial was 1–100,000 the dilution inactivation point; and in each case in which inactivation was reached only at 1–50,000, the highest value at which infection occurred was 1–10,000. The property which varies the least is thermal inactivation. Ten of the fourteen viruses have inactivation points within 6° of each other. With 3 of the remaining 4 viruses inactivation was obtained by heating at intervals of 3° to 5°. Had closer measurements been made this variation would probably have been reduced.

In regard to host range, the 14 viruses show many points of similarity, and yet each differs from the others. All infect one or more solanaceous and chenopodiaceous hosts. Local necrotic lesions are produced on tobacco by all but the T_1 strain of turnip mosaic (7). The virus of radish mosaic (13) is unique in that it becomes systemic in this host. Within the Cruciferae it is significant that only virus A, black ring virus, ring necrosis virus (6), the T_8 strain of turnip mosaic (7), the turnip virus of Chamberlain (2), and the radish mosaic virus (13) are widely pathogenic on subspecies of *Brassica oleracea*, although cabbage was the only subspecies tested with turnip virus 1 (3). Of the turnip mosaic viruses only the strain of Chamberlain (2) was pathogenic on wallflower; all were pathogenic on black mustard and rape except Tompkins' strain; only strain T_8 and T_9 (7) affected kohlrabi. Neither of the stock viruses (12) was pathogenic on members of *B. oleracea*. The mild stock mosaic virus affected wallflower.

In view of the points of similarity between these 14 viruses it seems more logical to consider them as strains of one virus group rather than

TABLE 2.—*Comparison of properties and host range of various crucifer viruses*

Virus	Dilution tolerance	Aging in vitro (hours at 20° C.)	Heating (°C. for 10 minutes)	Cabbage	Cauliflower	Broccoli	Kohlrabi	Kale	Brussels sprouts	Rape	Wallflower	White mustard	Black mustard	Turnip	Chinese cabbage	Dames violet	Annual stock	Tobacco	Nicotiana glutinosa	Cucumber	Currant tomato	Spinach	Swiss chard
Cabbage A (16)	1–10,000	192	60	S	S	S	S	S	S	S	S	S	S	S	S	S	S	L	L	O		S	S
Cabbage black ring (15)	1–1,000	72	59	S	S	S	S	S	S	S	S	S	S	S	S	S	S	L	L			S	S
Cabbage ring necrosis (6)	1–600	48	50	S	S	S	S	S	S	S	S	S	S	S	S	S	S	L	L	S		S	S
Turnip mosaic (9)	1–100,000³	72	54	S	S	S	S			S	O	O	O	S	S		S	O	L	O	O	O	
Turnip mosaic (11)	1–4,000	72	63	O	S	S	O	O	O	O	O	S	S	S	S	O	S	L	L	O	O	O	S
Turnip mosaic (8)	1–1,000	120	60	O	O	S	O	O	O	O	O	S	S	S	S	S	O	L	L		O	S	S
Turnip mosaic T₁ (7)	1–50,000	84	56	O	O	O	O	O	O	S	O	S	S	S	S	S	O	L	L	O	O	S	S
Turnip mosaic T₆ (7)	1–50,000	96	56	O	O	O	O	O	O	O	O	S	S	S	S	S	O	L	L	O	O	S	S
Turnip mosaic T₈ (7)	1–5,000	72	58	O	O	O	O	O	O	O	O	S	S	S	S	S	O	L	L		O	S	S
Turnip mosaic T₉ (7)	1–50,000	192	58	O	S	S	O	O	O	O	S	S	S	S	O	S	S	L	L	O	O	O	S
Severe stock mosaic (13)	1–4,000	144	60	S	O	O	O	O	O	O	S	S	S	S	S	S	S	L	L	O	O	S	S
Mild stock mosaic (12)	1–5,000	144	65	S	S	O	O	O	S	O	S	S	S	S	S	S	O	O	O	O	O	O	S
Rape mosaic (8)	1–7,000	384	68	S	S	S	O	S	S	S		S	S	S	S	O	S	O	L	O	O	O	S
Radish mosaic (3)	1–15,000	144	68	S	S	S	O	S	S	S		S	S	S	S	O	S	O	O	O	O	O	
Cauliflower mosaic (10)	1–1,500	360	75	S	S	S	O	O	O	S	O	S	S	S	S	O	S	O	O	O	O	S	
Cabbage B (16)	1–3,000	192	80	S	S	S	O	S	S	S	O	S	S	S	S	O	O	O	O	O	O	O	O
Broccoli mosaic (1)	1–3,000	96	75	S	S	S	O	S	S	S	O	O	S	S	S	O	S	L	L	O	O	O	
Chinese cabbage mosaic (14)	1–6,000		75	S	S	S	S	S	S	S	O	O	S	S	S	O	S	L	L	O	O	O	

¹ Values given are points at which inactivation occurred.
² S=Systemic infection; L=local reaction; C=no infection; no symbol=host not tested.
³ Inactivation was at 1–10,000 in 4 out of 5 trials.

as distinct viruses. The strain relationship of cabbage virus A and the black ring virus has been proved. These two viruses are considered as strains of turnip virus 1 (*3*) in view of the close similarities in symptoms, host range, and properties plus the fact that both virus A and turnip virus 1 were first obtained from the same area in southeastern Wisconsin. Turnip virus 1 is chosen as the type virus of the group because it was the first of the crucifer viruses for which critical property studies were made. Le Beau and Walker (*7*) have also considered turnip viruses T_1, T_6, T_8, and T_9 as strains of turnip virus 1. That the ring necrosis virus is a member of this group there is little doubt. It is the writers' opinion that the turnip viruses of Tompkins (*11*) and of Chamberlain (*2*), the two stock viruses, the rape mosaic virus (*8*), and the radish mosaic virus are also members of this group. The virus causing the ring spot disease of cabbage described by Smith (*9*) is very probably another strain of this group, but since no properties were given for this virus a close comparison with turnip virus 1 cannot be made.

Cabbage virus B, the cauliflower mosaic virus, the Chinese cabbage mosaic virus (*14*), and the broccoli mosaic virus (*1*) comprise a group that is quite distinct from the turnip virus 1 group in properties symptoms, and host range. These viruses are confined to the Cruciferae except for the local reactions of the Chinese cabbage virus on tobacco and *Nicotiana glutinosa*. Their characteristic symptoms are vein clearing and vein banding. The strain relationship of virus B and the cauliflower mosaic virus has been proved, and virus B is classed as a strain of cauliflower virus 1. In parallel inoculations to a few selected hosts in this laboratory the Chinese cabbage virus appeared to be quite similar to virus B and the cauliflower mosaic virus both in symptoms and in its tendency to become masked in cabbage at 28° C.

There appear, then, to be two groups of crucifer viruses, one group being represented by turnip virus 1 and the other group by cauliflower virus 1. This and other studies (*1, 3, 6, 7*) indicate that members of the turnip virus 1 group are favored by relatively high temperatures, while those of the cauliflower virus 1 group are favored by relatively low temperatures. With each group, masking tends to occur at unfavorable temperatures. Strains within one group may show variation in their individual temperature reactions. For example, on some hosts virus A is distinctly more severe than the black ring virus at 28° and 16° C. On other hosts the two are practically indistinguishable at high temperatures, but at low temperatures their reactions are widely different, owing chiefly to the increased severity of the black ring symptoms. It is apparent, then, that the black ring virus is favored by lower temperatures than some other members of this group. Little differential temperature reaction has been observed among members of the cauliflower virus 1 group.

SUMMARY

When the reactions of cabbage virus A, cabbage black ring virus, cabbage virus B, and cauliflower mosaic virus on cabbage were studied at various temperatures, it was found that they fell into two distinct groups.

In the turnip virus 1 group, containing cabbage virus A and the black ring virus, the progress and severity of disease development varied directly with the air temperature to which the plants were exposed, symptoms (except for necrosis with the black ring virus) being most severe at 28° C. and mildest at 16°. The characteristic symptom of both viruses was a coarse chlorotic mottle accompanied by leaf malformation. However, some marked differences between the reactions of the two viruses to temperature were observed. Symptoms of virus A were distinctly more severe than those of the black ring virus at 28° and 24°, but at 20° and 16° the exact reverse was true.

In the cauliflower virus 1 group containing cabbage virus B and the cauliflower mosaic virus, symptom intensity was also found to be directly proportional to the air temperature. However, in contradistinction to the turnip virus 1 group, symptom intensity increased with decrease in temperature and complete masking occurred at 28° and 24°. The change in reaction with change in temperature was exactly parallel with both viruses. The characteristic symptoms of these viruses were chlorotic vein clearing and vein banding.

When either virus A or the black ring virus occurred in cabbage together with either virus B or the cauliflower mosaic virus, the resulting disease reaction was more severe than that produced by either virus alone. The increased severity of symptoms was so pronounced at 28° and 24° that they appeared as those of an entirely different disease. At low temperatures the activity of virus A or the black ring virus was so reduced that combination symptoms agreed very closely with those of virus B or of the cauliflower mosaic virus. In such combinations the black ring virus reacted very similarly to virus A, and the cauliflower mosaic virus reacted similarly to virus B. When the temperature at which plants infected with a virus combination were growing was reversed from high to low, or vice versa, there resulted a corresponding reversal in symptom type.

At high temperatures symptoms produced by virus A on *Brassica oleracea* var. *gemmifera*, *Nicotiana rustica*, and *N. multivalvis* were practically indistinguishable from those produced by the black ring virus, but at low temperatures the reactions of the two viruses on these hosts were markedly different. A striking effect of temperature on the type of symptoms produced by these two viruses on *N. glutinosa* is described.

By use of the differential reaction between virus A and the black ring virus on the hosts mentioned above, virus A was shown to effectively immunize cabbage against infection by the black ring virus. Likewise, the black ring virus protected cabbage plants against infection from virus A, as measured on *Solanum integrifolium*, a host which was completely differential for virus A. Both virus A and the black ring virus are classed as strains of *turnip virus* 1.

At both high and low temperatures the reaction of the cauliflower mosaic virus was much more severe than that of virus B on such hosts as *Brassica pekinensis*, *B. nigra*, *B. napus*, *B. arvensis*, and *B. campestris*. By the use of the differential reaction on *B. pekinensis*, virus B was shown to immunize cabbage against infection by the cauliflower mosaic virus. Virus B is classed as a strain of *cauliflower virus* 1.

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MEASUREMENTS OF THE VOLATILE PRODUCTION OF APPLES [1]

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INTRODUCTION

The investigations of Brooks and Cooley (*1, 2*)[2] and Brooks, Cooley, and Fisher (*3, 4, 5*) on apple scald control with oiled wrappers and shredded oil paper indicate that organic vapors arising from apples are directly responsible for the development of this physiological disorder. However, there is no satisfactory evidence at present to show which one or ones of the many volatile constituents emanating from apples are responsible for storage scald.

If apple volatiles are truly responsible for the development of apple scald, quantitative measurements of the organic vapors arising from apples in the fall might indicate the relative amount of scald that one could expect at any given stage of maturity and set of storage conditions.

Since it is generally considered that the organic gaseous products arising from apples are directly responsible for apple scald, it seemed desirable to determine their magnitude. Measurements of volatile emanations and respiration were accordingly made on McIntosh apples (*Malus pumila* Mill.) picked at regular intervals through two harvesting seasons. The influence of temperature, storage atmosphere, and vapors from ripe apples on the rate of respiration and volatile production were also studied. Special precautions were taken to make sure that vapors from previously harvested fruit did not come in contact with the experimental apples.

REVIEW OF LITERATURE

The most complete analysis of the odorous constituents arising from apples was made by Power and Chesnut (*25*). By suitable chemical procedures they found amyl ester of formic, acetic, caproic and caprylic acids, acetaldehyde, and ethyl alcohol. Later, they (*26*) discovered geraniol, an aliphatic terpene alcohol, in the peel of McIntosh apples. It is of interest that they were unable to detect the presence of amyl valerate which is generally known as "apple oil."

Probably the most widely known of the organic metabolic gases arising from fruits is ethylene. Elmer (*6*) noticed that apple vapors

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inhibited the sprouting of potatoes. Later, Smith and Gane (27) found that apple emanations greatly decreased the growth of pea seedlings. By using large numbers of absorbents they noted that bromine, ozone, fuming nitric acid, and fuming sulfuric acid removed the active agent from apple vapors. All of these chemicals react readily with unsaturated hydrocarbons. Eventually, Gane (7), Hansen and Christensen (16), and Niederl, Brenner, and Kelley (24) identified ethylene as a constituent of vapors emanating from apples. Hansen and Christensen (16) found no acetylene, propylene, or butylene. Niederl, Brenner, and Kelley (24) were also unable to find propylene or butylene. Consequently, the influence of apple vapors as observed by Elmer (6) and the epinastic effects noted by Gane (10) are generally considered to be due to ethylene.

Quantitative measurements of total volatiles arising from apples have been made by Gane (11) and Kidd and West (21) by employing a complete combustion technique. Large differences were found in the volatile respiration ratios among different varieties of apples. Gane (11) shows that these differences in ratios are due mainly to variations in the production of organic vapors. Kidd and West (21) observed that as the temperature was lowered from 15° to 3° C. the rate of respiration and volatile production dropped markedly. Their data with Bramley's Seedling apple indicate that although the curve in the production of volatiles follows respiration rather closely, it lags behind respiration and reaches its peak a number of days after the respiratory climacteric is reached.

Recently, Gerhardt and Ezell (14) measured the volatiles from Golden Delicious apples by absorbing the emanations in concentrated sulfuric acid and subsequently oxidizing them with ceric sulfate. At 65° F. the climacteric in respiration preceded the peak in volatile production by 6 days. After this peak in organic vapors had been reached, the rate dropped rapidly. It was later realized by Gerhardt (13) that ethylene was not absorbed appreciably by concentrated sulfuric acid.

Acetaldehyde is one of the most prevalent of organic emanations from fruit and has been measured frequently from apple and pear tissue. Harley and Fisher (18), Harley (17), and Gerhardt and Ezell (15) have stressed the importance of acetaldehyde as the causal agent of scald and core break-down of pears. Gerhardt (13), working with apple tissue, found that acetaldehyde accumulated in areas affected by soft scald. Miller (22) however, points out that—

* * * high acetaldehyde content of apple peel may be due in part to its production by cells ruptured in the paring process, but there is also a tendency for the acetaldehyde to accumulate in peel affected either by mechanical injury to the cells or by abnormal physiological conditions of the fruit.

The quantities of ethylene arising from apples is small, although there is undoubtedly much variation among varieties. Gane (8) estimates that approximately 1cc. of ethylene is produced by an apple in its entire life. Nelson (23) has shown that at 20° C. McIntosh apples liberate their largest quantities of ethylene about 5 days after the respiratory peak. Their maximum output was about 2.0 mg. of ethylene per kilogram of fruit per day at this temperature.

The influence of ethylene on the rate of respiration of preclimacteric fruit is well known. Gane (12) and Kidd and West (19, 20), working

with bananas and apples, respectively, noted that the climacteric in respiration could be brought about by low concentrations of ethylene. Gane (*12*) found that bananas which were in their postclimacteric phase were uninfluenced by ethylene so far as the respiratory rate was concerned.

Kidd and West (*19*) and Smock (*29*) have been able to induce the climacteric in sunripe apples by passing vapors from ripe fruits over the immature ones. The vapors from ripe apples, however, were unable to influence the rate of respiration of apples which were already respiring at an accelerated rate.

METHODS

The procedure employed for the measurement of apple volatiles was one devised by Gerhardt and Ezell (*14*) with a few modifications. The absorbent used was 35 ml. of concentrated sulfuric acid (specific gravity, 1.84) placed in a suitable absorption tower. The towers were made from 2-foot sections of 24-mm. pyrex glass tubing, stoppered into 500-ml. suction flasks. The towers were then about half filled with pyrex glass beads (2 to 6 mm). The air containing the apple vapors passed through the towers during the 20- or 24-hour determinations at the rate of approximately 28 liters per hour. After the absorption period was completed, the tower was removed from the suction flasks and the beads were allowed to fall into the flasks. The sides of the tower were washed down with distilled water, care being taken to collect all washings in the suction flask. Then a known volume of standard ceric sulfate was added and the solution was made up to 250 to 300 ml. with distilled water. The flasks were then placed in an autoclave and allowed to remain there from 6 to 8 hours at 15 pounds pressure (approximately 121° C.). At the end of that time the excess ceric sulfate was titrated with approximately 0.1 N ferrous sulfate, orthophenanthroline ferrous sulfate being used as the indicator (*28*). The exact normality of ferrous sulfate was determined daily by standardizing it against the previously standardized ceric sulfate (*28*) which is exceedingly stable (*31*).

Gerhardt and Ezell (*14*) reported that their method was satisfactory after duplicating their results very closely when known quantities of acetaldehyde were oxidized. Since they knew that acetaldehyde was an organic volatile arising from fruits, they believed that the apple emanations could be determined by the same procedure. It was thought, however, since esters are also produced by apples, that it might be wise to standardize their procedure against an ester. Ethyl acetate was selected as the ester to be used.

A diagram of the apparatus in which ethyl acetate was placed is shown in figure 1. With the mercury in the positions indicated in the diagram, the apparatus was weighed to the nearest tenth of a milligram on an analytical balance. Then a sample of ethyl acetate vapor was drawn off by passing air through this container in the direction indicated in figure 1, and absorbed in the tower containing the sulfuric acid. As the air moved through the container for the ester, the mercury was forced up into the side nipples. If any of the mercury splashed past the nipples it was either caught in the trap or in the bulb containing the volatile liquid. Except for necessary

rubber connections all tubing was either of glass or copper. After an hour or two the container holding the ethyl acetate was removed from the system, and by tipping this volatile holder properly the mercury was allowed to flow back into the positions shown in figure 1. The container was immediately reweighed and the loss in weight represented the ethyl acetate absorbed by the sulfuric acid. Known volume of ceric sulfate were added as previously described and after digestion and titration, the milligrams of ceric sulfate reduced per milligram of ester oxidized were calculated.
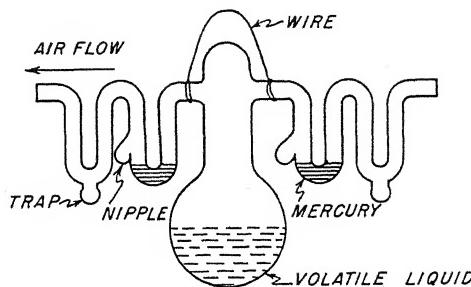
FIGURE 1.—Diagram of container used to measure the weight of ethyl acetate lost to an absorption tower.

The influence of the length of the digestion period and temperatures during digestion are given in tables 1 and 2. From the data shown in table 1 it can be seen that no readily reproduceable reduction-oxidation values are obtained by boiling for periods up to 21 hours. Gerhardt and Ezell (*14*) found that acetaldehyde could be completely oxidized in 2 hours at 98° C. However, by digesting this solution in an autoclave at 15 pounds' pressure at intervals of 2 hours and titrating the excess ceric sulfate after each period, it was possible to determine the time required to completely oxidize this ester at this temperature. By comparing the results presented in table 1 with those in table 2 it can be seen that after 6 hours at 15 pounds' pressure the reduction-oxidation values were much greater than those resulting after 15 hours of boiling.

The variations in the reduction-oxidation ratios are not considered significant in view of the crudeness of the apparatus used. It was not the author's desire to determine the absolute reduction-oxidation ratio for ethyl acetate, but rather to find how long a period and how high a temperature would be necessary to oxidize the apple emanations which are absorbed in sulfuric acid.

TABLE 1.—*Influence of time and temperature on the oxidation of ethyl acetate with ceric sulfate*

Ethyl acetate (milligrams)	Oxidation		Ceric sulfate (0.1060 N) used	Ceric sulfate reduced	Reduction-oxidation ratio
	Time	Temperature			
	Hours	°*C.*	*Milliliters*	*Milligrams*	
86.7	15	88	50	1,353.5	15.6
90.3	15	88	50	1,284.9	14.2
130.1	15	88	50	1,599.8	12.3
93.6	15	98	50	1,509.4	16.1
117.8	15	98	50	1,765.1	15.0
114.5	21	98	75	2,058.2	18.0

TABLE 2.—*Influence of time and temperature on the oxidation of ethyl acetate with ceric sulfate under 15 pounds pressure*

Ethyl acetate (milligrams)	Oxidation		Ceric sulfate, 0.1060 N., used	Ceric sulfate reduced	Reduction-oxidation ratio
	Time	Temperature			
	Hours	*°C.*	*Milliliters*	*Milligrams*	
56.9	2	121	50	1,523.1	26.77
	2	121	25	73.4	1.29
	2	121	25	8.6	.15
Total 56.9	6	121	100	1,605.1	28.21
59.1	2	121	50	1,527.1	25.84
	2	121	25	144.2	2.44
	2	121	25	12.0	.20
Total 59.1	6	121	100	1,683.3	28.48
53.9	2	121	50	1,467.3	27.22
	2	121	25	50.8	.94
	2	121	25	5.7	.11
Total 53.9	6	121	100	1,523.8	28.27
64.8	2	121	50	1,687.9	26.05
	2	121	25	122.6	1.89
	2	121	25	10.6	.16
Total 64.8	6	121	100	1,821.1	28.10

After observing the results shown in tables 1 and 2 it seemed quite probable that complete oxidation of apple volatiles by ceric sulfate might not take place after 2 hours of boiling. The results shown in table 3 were obtained after oxidizing the organic vapors arising from one-half bushel of ripe McIntosh apples at room temperature.

TABLE 2.—*Influence of time and temperature on the oxidation of ethyl acetate vapors with ceric sulfate*

Oxidation		Ceric sulfate, 0.1261 N, used	Ceric sulfate reduced per day	Ceric sulfate reduced per kilogram of fruit per day
Time (hours)	Temperature			
	°C.	*Milliliters*	*Milligrams*	*Milligrams*
2	121	50	996.0	135.8
2	121	25	136.1	18.6
2	121	25	13.3	1.8
2	121	25	6.6	.9
2	121	25	0	0
Total	10		150	1,152.0	157.1
2	98	25	368.5	50.2
2	121	25	667.3	90.9
2	121	25	56.4	7.7
2	121	25	10.0	1.4
2	121	25	6.6	.9
Total	10		125	1,108.8	151.1
2	98	25	368.5	49.0
2	121	25	640.8	85.1
2	121	25	49.8	6.6
2	121	25	10.0	1.3
2	121	25	10.0	1.3
Total	10		125	1,079.1	143.3

From the results obtained it seems quite clear that boiling for 2 hours is hardly sufficient for complete oxidation of apple volatiles. The data also indicate that a large proportion of the vapors arising from ripe apples was not acetaldehyde. All subsequent determina-

tions of apple volatiles in this study were made after they had been held at 15 pounds pressure from 6 to 8 hours. The data presented in table 4 indicate that ceric sulfate is sufficiently stable to stand such treatment.

It is realized that ethylene is not appreciably absorbed by concentrated sulfuric acid as used in these determinations.

The apples from which volatile measurements were made were placed in 2-gallon jars in 1941 and in 18-liter jars in 1942. These jars were placed in a constant-temperature bath. The 2-gallon jars held 10 apples and the fruit in them weighed between 1 and 2 kg. In the 18-liter containers anywhere from 40 to 70 apples were used, and these fruits weighed generally about 7 kg. The air passing over the fruit was brought to the desired temperature by having it go through coils of copper tubing, immersed in a water bath at constant temperature before it reached the fruit. The air moved constantly over the apples at the rate of about 28 liters per hour. In 1941 most of the determinations lasted 24 hours, whereas, in 1942, 20-hour runs were made.

TABLE 4.—*Influence of time and temperature on the stability of ceric sulfate*

Oxidation		Ceric sulfate used	Normality of ceric sulfate
Time (hours)	Temperature		
	°C.	*Milliliters*	
8	Room	25	.1200
8	Room	25	.1199
8	Room	25	.1200
8	121	25	.1197
8	121	25	.1197
8	121	25	.1195

Since activated lignite charcoal (Darco) was found to be very effective in absorbing the same vapors from apples that react with sulfuric acid, 50 to 80 gm. of this granulated charcoal was used, in 1941, to cleanse the air before it passed over the experimental fruit. Towers of concentrated sulfuric acid were not used to cleanse the air because it was not possible, with the mercury pump employed, to cause air to flow through two columns of sulfuric acid at once. In 1942, when the weight of the fruit was increased many times, it was found that the air, in a room free of apples and well ventilated with outdoor air, was sufficiently free of material oxidized by ceric sulfate to make special precautions unnecessary. The amount of material found in this air generally amounted to 1 or 2 mg. of ceric sulfate reduced per kilogram of fruit per day. Since the quantities in the air were small and quite constant, they were disregarded. Gerhardt and Ezell (*14*) found only 16 mg. of ceric sulfate to be reduced after collecting the volatiles in fresh air for 6 days at the rate of 10 liters per hour.

The volatile determinations made at 32° and 40° F. throughout the storage season of 1941 and 1942 were taken from 2 bushels of apples placed in air-tight steel drums. The volatile production for each day was measured.

In 1942 aldehyde measurements were made. The aldehydes were removed from the absorption train by placing concentrated solutions of sodium bisulfite in towers similar to those containing the sulfuric

acid. The towers were arranged in series so that the aldehydes reacted with sodium bisulfite before they could be absorbed by the concentrated sulfuric acid. The method used for measuring aldehydes was To-moda's (*30*) modification of Ripper's procedure. Tomoda's method was modified by making a very strong solution of sodium bisulfite (250–300 gm. per liter). This was essential because the determinations lasted 20 hours and unless a large excess of sodium bisulfite was present at the start of each run, there would be no excess of bisulfite at the end of the measurement. When a large excess of sodium bisulfite was present at the end of a determination, it was removed by passing aldehyde-free air through the tower until only a slight excess remained. This point could be determined by inhaling some of the vapors arising from the tower. When only a faint odor of sulfur dioxide could be detected, a reasonably small excess of sodium bisulfite was present, and one could proceed as outlined by Tomoda (*30*). The concentration of aldehydes from apples harvested in 1942 was found to be so small that 0.01 N iodine or slightly less was used in the titrations. The aldehyde content was calculated as milligrams of acetaldehyde per kilogram of fruit since it is known to be a constituent of apple vapors.

In 1941 the respiration measurements [3] were made on the same days as the volatile determinations, but on similar lots of fruit; in 1942 the volatile and respiration measurements were made on the same lots of fruit. The volatile determinations lasted 20 hours, and the respiration runs were carried out during the remaining 4-hour period.

All the fruits used in these experiments were picked from the orchard at Cornell University, Ithaca, N. Y.

Further details pertaining to individual experiments will be taken up as they are discussed in the text.

<center>RESULTS</center>

<center>INFLUENCE OF STORAGE TEMPERATURES ON RATE OF VOLATILE PRODUCTION</center>

McIntosh apples were picked on September 19, 1941, and two 2-bushel lots of uniform and blemish-free fruits were composited. One lot was placed in a 55-gallon steel drum at 32° F.; the other was placed in a similar container at 40°. Air passed through granular activated charcoal was drawn over the fruit at the rate of 28 liters per hour. The volatiles arising from the apples were measured daily during the storage period for 2 weeks after they were removed from storage, during which time they were held at 74° F. The data for the experiment are presented in figure 2.

Each point in *A* and *B* represents the average daily rate of volatile production for the week previous. The points on the cumulative curves shown in *C* represent the total amount of ceric sulfate reduced per kilogram per week until the apples were removed to 74° F. At 74° the daily production of apple emanations was plotted.

The average rate of volatile production at 40° F. was generally about twice that at 32° throughout the storage season. At 32° the rate of production fell very gradually until January and then rose to a peak in early February. The climacteric in respiration occurred
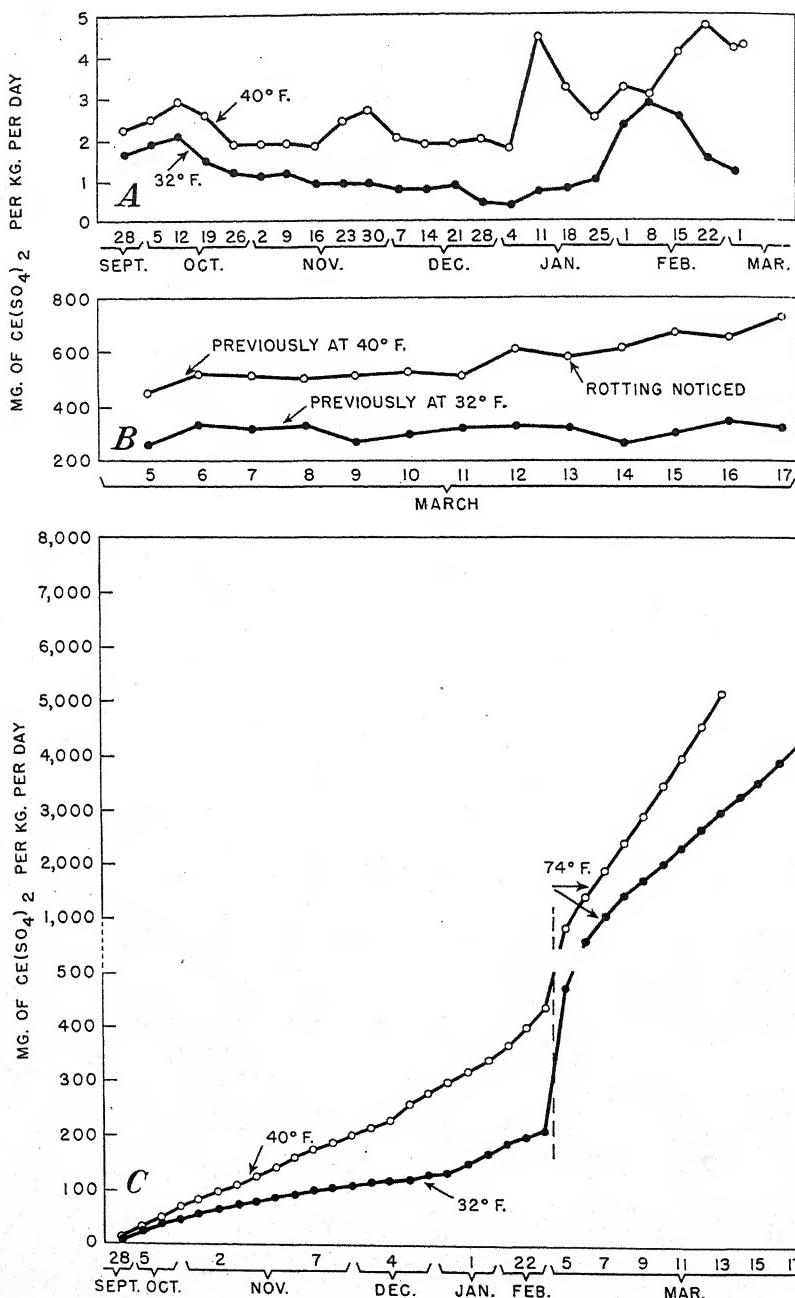
FIGURE 2.—Rate of volatile production from McIntosh apples picked September 19, 1941: *A*, Apples stored at 32° and 40° F.; *B*, apples held at 74° after removal from storage at 32° and 40°; *C*, cumulative curves showing total amount of ceric sulfate reduced per kilogram per week until apples were removed from storage to a temperature of 74°; and thereafter, the daily volatile production until the conclusion of the experiment in March 1942.

in December. The apples held at 40° evolved organic vapors at a rather constant rate until January, at which time the rate rose rather sharply and then continued to increase gradually, except for the period from January 4 to 25, until the fruits were removed from storage in March. The quantity of measurable material arising from the apples at both 32° and 40° was less than 1 percent of the volatile emanations produced by the same fruit at 74°. However, the fruit which had previously been stored at 32° was marketable for a longer period after removal from storage, and as shown by the cumulative curves in figure 2, evolved about as much total volatile material before becoming unmarketable as the apples formerly held at 40°.

INFLUENCE OF MATURITY AND RIPE-APPLE VAPORS ON RATE OF RESPIRATION AND VOLATILE PRODUCTION
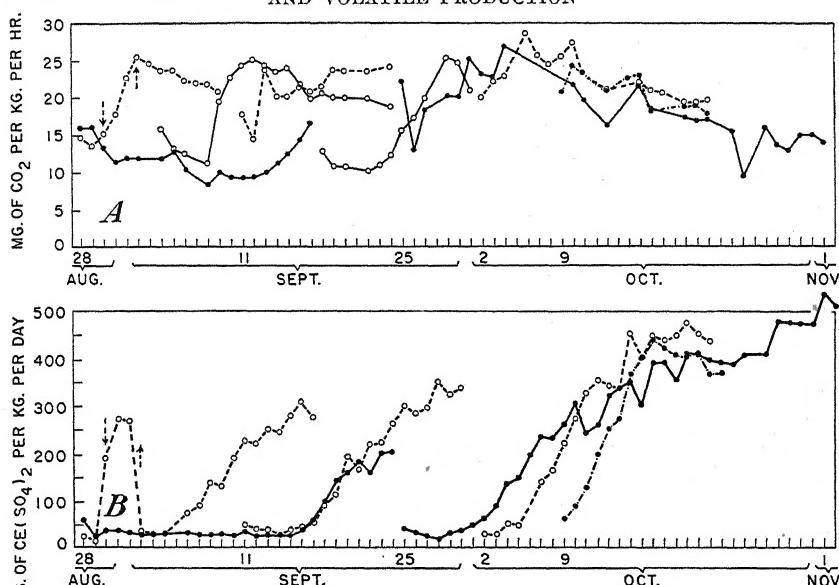
FIGURE 3.—Influence of maturity and ripe apple vapors on rate of respiration and volatile production of McIntosh apples picked at different dates in 1941 and held for different periods at 74° F., as indicated by: *A*, Carbon dioxide produced per kilogram per hour; and *B*, ceric sulfate reduced per kilogram per day. Each curve represents data obtained from apples immediately after they were picked. The broken line, commencing August 28, represents the treated apples, and the solid line the untreated apples. Downward-pointing arrows indicate start of 2-day treatment with vapors from ripe McIntosh apples; upward-pointing arrows, the end of this period.

1941 EXPERIMENT

The data gathered in 1941 showing the influence of maturity on the rate of respiration and volatile production of McIntosh apples are presented in figure 3. There are also some data illustrating the influence of ripe apple vapors on preclimacteric fruit.

The curves for volatile production (fig. 3, *B*) follow the respiratory curves (fig. 3, *A*) quite closely. However, the rise in the rate of production of odorous emanations lags behind the preclimacteric rise in respiration. With the organic vapors there does not seem to be any

sharply defined peak. The amount of volatiles produced continued to rise practically until the experiment ended, although the rate of increase became less pronounced with time. The rate of respiration, on the other hand, rose to a rather well defined maximum and then fell gradually.

In general, it can be stated that the number of days preceding the climacteric rise in respiration and volatile production became less as the date of harvesting was delayed. In fact, the apples picked on October 9 were already respiring at their maximum rate and the odorous emanations reached a climax shortly thereafter.

The influence of vapors from six ripe Early McIntosh apples was tested on one-half bushel of McIntosh apples harvested on August 27. The arrows pointing downward indicate the start of the period during which these ripe apple vapors were drawn over preclimacteric fruit, and the upward-pointing arrows indicate the end of this 2-day treatment. The broken line starting on August 28 represents the treated lot; the solid line represents the control. The volatile measurements during the 2-day period include the vapors from the ripened fruit as well as those from the McIntosh apples. The vapors from the ripe fruit greatly shortened the preclimacteric period of respiration and volatile production.

1942 Experiment

In 1942 the influence of maturity and the effect of vapors from ripened Early McIntosh apples on the rate of respiration and volatile production of McIntosh apples was again studied, but in that year part of the total organic material was measured as acetaldehyde. The data are presented in figures 4 and 5. A comparison of the results in figure 4, *E*, with those in figure 3, *B*, shows that the quantities of ceric sulphate reduced were very much higher in 1941 than in 1942. Since Gerhardt and Ezell (*14*) found that 1 mg. of acetaldehyde reduced about 51 mg. of ceric sulfate, it can be seen that even if the aldehydes are taken into account 15 or 20 mg. of ceric sulfate were reduced per kilogram of fruit per day when the maximum quantities of aldehydes were being produced. The amount of ceric sulfate reduced per kilogram of fruit per day in 1941 rose to between 300 and 450 mg. within 2 weeks after harvest, except for the first picking, whereas in 1942 between 40 and 300 mg. of ceric sulfate were reduced. Only after the last two pickings in 1942 did the quantity of volatile material reduced approach that evolved in 1941 for a similar length of time. The fact that determinations were of 20 hours' duration in 1942 as compared to 24 hours in 1941 would not account for these differences in volatile production. The maximum rates of respiration were also lower in 1942 than in 1941. It was noted that all the varieties studied for apple scald in 1941-42 were injured earlier and much more severely than similar apples in 1942-43.

The shape of the curves for 1942 are quite similar to those for 1941, and the same general conclusions may be drawn from them. Toward the end of the harvesting period the preclimacteric phase became shorter insofar as respiration and volatile production were concerned. Eventually apples were picked (October 9) which were already in their postclimacteric phase of respiration. The increase in the rate of volatile production lagged behind the preclimacteric rise in respira-
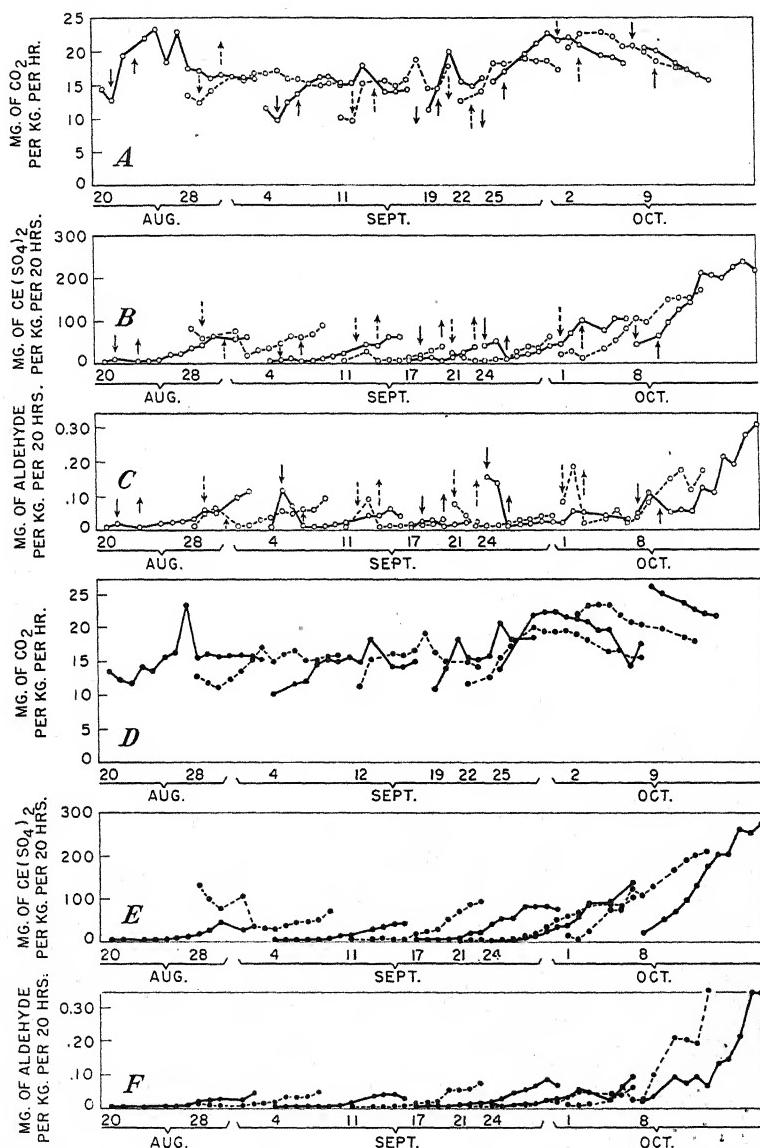
FIGURE 4.—Influence of maturity and effect of vapors from ripened Early McIntosh apples on the rate of respiration and volatile production of McIntosh apples, picked at different dates in 1942 and held for different periods at 74° F. *A*, Carbon dioxide given off per kilogram per hour; *B*, ceric sulfate reduced per kilogram in 20 hours; and *C*, aldehyde produced per kilogram in 20 hours. Downward-pointing arrows indicate start of 2-day treatment with vapors from ripened McIntosh apples; upward-pointing arrows, the end of this period. Influence of maturity and effect of vapors from unripened McIntosh apples on the rate of respiration and volatile production of McIntosh apples, picked at different dates in 1942, and held for different periods at 74° F. *D*, Carbon dioxide given off per kilogram per hour; *E*, ceric sulfate reduced per kilogram in 20 hours; and *F*, aldehyde produced per kilogram in 20 hours. Each curve, *A–F*, represents data obtained from apples immediately after they were
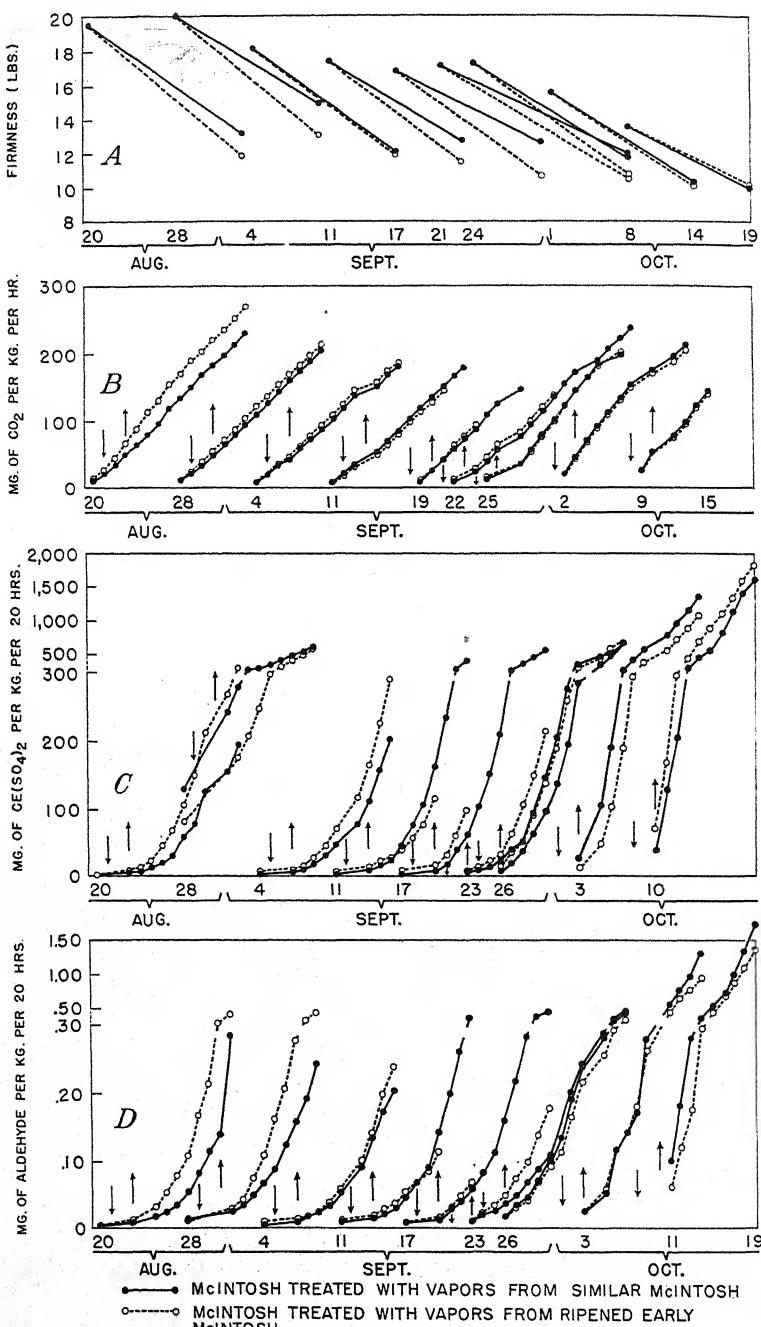
FIGURE 5.—Comparison of firmness, cumulative respiration, and cumulative volatile production from McIntosh apples treated with vapors from ripened and unripened apples at 74° F. *A*, Firmness; *B*, CO_2 given off per kilogram per hour; *C*, ceric sulfate reduced per kilogram in 20 hours; *D*, aldehyde produced per kilogram in 20 hours. Downward-pointing arrows indicate start of

The graphs in figure 4, *A–C*, represent the rate of respiration and volatile production of McIntosh apples treated with vapors from six ripened Early McIntosh apples for a period of 2 days as indicated by arrows. The graphs in figure 4, *D–F*, show the rate of respiration and volatile production from similar McIntosh apples but treated with vapors from six McIntosh apples harvested at the same time. Figure 5 presents in cumulative form the data given in figure 4 together with the results on the firmness of the flesh at the start and conclusion of each determination.

A careful study of figures 4 and 5 shows that except for the stimulatory effect of the vapors from ripe Early McIntosh apples after the first two pickings (August 20 and 28) and after the sixth picking (September 21), probably no significant effect on respiration or volatile material was produced. It is true that the amount of volatile material produced by McIntosh apples after treatment with vapors from ripened Early McIntosh varies from the production of organic material by similar untreated apples for the last one or two pickings (October 1 and 8), but these differences appear to have been due to natural variations present at the beginning of the experiment rather than to any stimulatory effect of other apple vapors. One fact which seems quite clear is that a stimulatory effect on the rate of respiration or volatile production from ripe-apple vapors occurs when the exposed fruit is in the phase preceding the preclimacteric rise in respiration.

The pressure data are not in complete agreement with the respiration or the volatile data. One would expect that those fruits that respire at a similar rate would become softer at a corresponding rate. This was not always the case. However, those apples that respired at a significantly higher rate were always softer.

RATE OF RESPIRATION AND VOLATILE PRODUCTION FOLLOWING REMOVAL OF APPLES FROM COLD AND MODIFIED ATMOSPHERE STORAGE

An experiment was made in 1942 to determine the rate of respiration and volatile production of McIntosh apples immediately after they were removed from storage. The determinations were made at 74° F. from fruit which had been held previously at 32° in air and at 40° with 5 percent carbon dioxide and 2 percent oxygen until January 2, 1942. The data are presented in figure 6.

The respiration data show that the apples held at 32° F. had already passed through their climacteric rise and were in the postclimacteric phase. The respiratory rate of the apples which came from the controlled atmosphere chamber rose to a peak shortly after they were removed to 74°. These curves indicate that apples respire more slowly after removal from controlled atmosphere storage at 40° than do similar apples after removal from air storage at 32°. The apples stored at 32° were riper, softer, and became unmarketable sooner than the apples held in the controlled atmosphere chamber. Apples brought from a storage room low in oxygen and high in carbon dioxide often seem to lack aroma. The quantitative measurements of volatile emanations show that they are less odorous. Although the rate of respiration gradually decreased during the postclimacteric phase, the rate of volatile production continued to rise even after the apples were unmarketable.
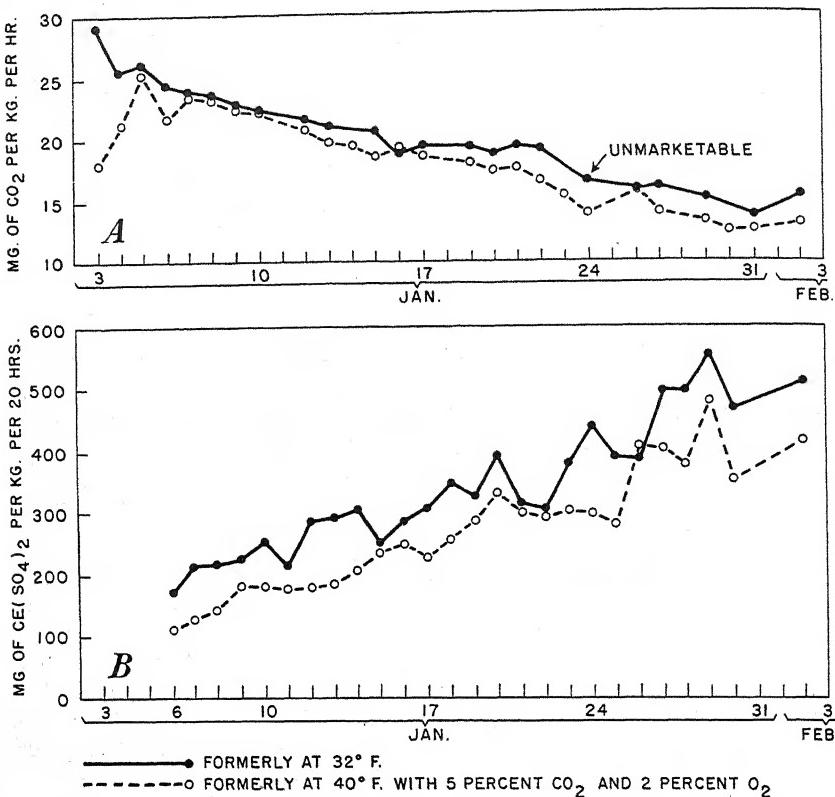
FIGURE 6.—Rate of respiration and volatile production of McIntosh apples at 74° F. after storage at 32° in air and at 40° with 5 percent carbon dioxide and 2 percent oxygen, as shown by: *A*, carbon dioxide produced per kilogram per hour; *B*, ceric sulfate reduced in 20 hours.

DISCUSSION

No one has yet shown which fraction or fractions of apple emanations are related to the scald diseases. Consequently, gross measurements of the apple volatiles reacting with concentrated sulfuric acid might appear to be of little value, for it is conceivable that the quantities of various volatile fractions emanating from apples might vary greatly even though the total volatile production remained constant. Data presented above show that the rate of volatile emission follows the rate of respiration quite closely. These results are similar to those of Kidd and West (*21*) and Gane (*9*, *11*). So far as individual fractions are concerned, the data herein presented show that as those volatiles which are absorbed by sulfuric acid increase, so does the production of aldehydes. Nelson (*23*) has shown that the rate of ethylene emission from McIntosh apples rises as the rate of respiration increases. From the evidence now at hand it seems likely that changes in the rate of production of separate fractions from apples are a result of fluctuations in the rate of respiration, for the peak in the production of these emanations lagged behind the climacteric in respiration. Consequently, comparisons of the total volatiles produced might at

least indicate the relative quantity of scald gases being evolved. The possibility that these differences in apple emanations may be correlated with the time at which scald appears and its severity was noted in 1941–42 and 1942–43. In 1941 the McIntosh apples held at 74° F. evolved a great deal more volatile material than did similar apples in 1942. The scald in 1941 was visible sooner and was much more severe than in 1942. Since the volatile curves resemble the respiratory ones, it might be possible to predict the severity of scald by comparing the carbon dioxide measurements from year to year during the fall. Although there were differences in respiration between 1941 and 1942, they were relatively small when compared to the variations in volatile production between these 2 years.

SUMMARY AND CONCLUSIONS

This paper reports the results of a study to determine the influence of various factors on the production of volatile material from apples and on the rate of their respiration.

McIntosh apples stored at 32° F. were found to evolve about half as much volatile material as similar apples held at 40°. When the fruits were removed from storage and placed at 74°, they emitted more organic vapors in 1 day than they had during 5 months in storage. Apples which had previously been held at 40° continued to evolve more volatile material than fruits which were formerly held at 32°.

Apples stored in an atmosphere of 5 percent carbon dioxide and 2 percent oxygen at 40° F. respired and produced volatiles at a slower rate, when removed to room temperature, than similar fruit previously stored at 32° in air.

The quantity of volatile material arising from McIntosh apples harvested in 1941 and held at 74° F. was found to be much higher than that from similar fruit harvested in 1942. As the severity of scald was much more pronounced in 1941–42 than in 1942–43, it has been suggested that differences in apple emanations from one year to the next may be correlated with the amount of apple scald that will occur during the storage season for any given stage of maturity and set of storage conditions.

Apples which are in the preclimacteric stage may be stimulated by an active agent (ethylene) so that the rate of volatile production as well as respiration is increased.

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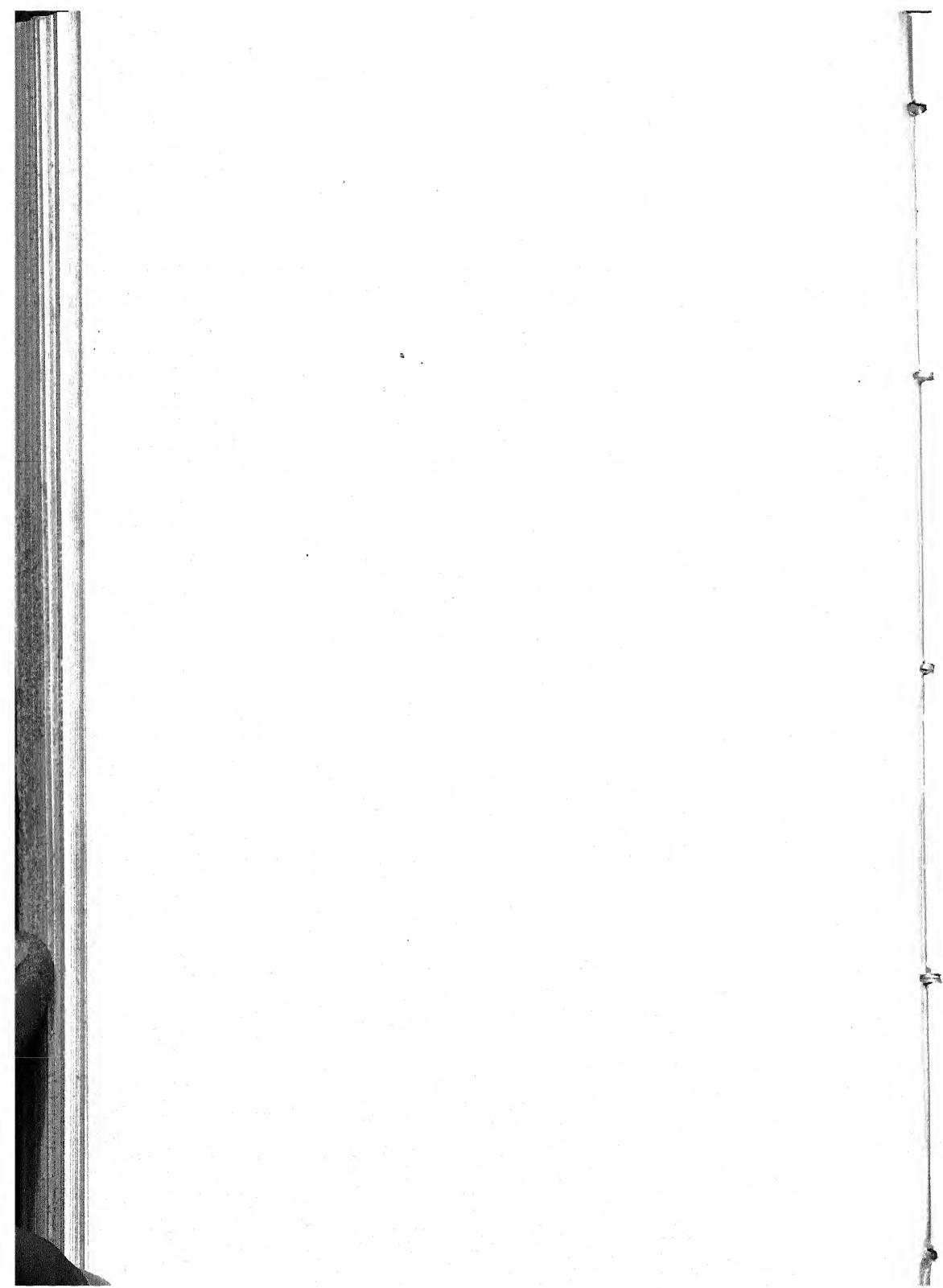
THE REMOVAL OF ORGANIC EMANATIONS FROM THE ATMOSPHERE SURROUNDING STORED APPLES [1]

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INTRODUCTION

The work of Brooks and his associates (*1, 2, 3, 4, 5*) [2] indicated that the cause of apple scald is closely related to the organic emanations arising from the apples themselves. Smock and Southwick (*22*) further found that one lot of apples may hasten the development of scald on another lot in storage, and Smock (*21*) showed that ethylene arising from postclimacteric apples may shorten the storage life of preclimacteric apples by as much as 25 percent.

Since both rate of ripening and scald development appear to be related to the presence of organic apple emanations, an atmospheric washing agent was sought which would remove the emanations and so improve the quality and lengthen the life of stored applies. Oiled paper used as wraps about the fruit or shredded and mixed with them is the most practicable method of controlling scald at the present time. However, in the northeastern apple-growing sections this method has often given unsatisfactory control. The use of oiled paper also involves considerable labor and expense. Consequently, a more effective and simpler way of obtaining scald control is needed.

To be of practical value to commercial storage operators any material employed to "air-condition" a storage atmosphere should be inexpensive, readily obtainable, easy to use, and harmless to the fruit. Therefore, emphasis has been placed on some compounds which probably could be used commercially, if found satisfactory from the standpoint of their ability to remove volatile materials.

Measurements have been made of the absorptive and adsorptive capacity of various materials for vapors of ethyl acetate, those apple emanations which react with concentrated sulfuric acid, and the ethylene in ripe apple vapors. Respiration measurements were made in conjunction with most of the volatile measurements. [3]

REVIEW OF LITERATURE

An analysis of apple emanations by Power and Chesnut (*16, 17*) showed the presence of amyl esters of formic, acetic, caproic, and caprylic acids, ethyl alcohol, acetaldehyde, and geraniol. Ethylene has been identified, also, as a constituent of apple emanations by many other investigators (*6, 8, 14, 15*).

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[1] Received for publication January 4, 1944.
[2] Italic numbers in parentheses refer to Literature Cited, p. 313.
[3] These measurements were made by Dr. R. M. Smock, associate professor of pomology at Cornell University, and have already appeared in published form (*21*). They are presented here, with his permission, merely to show the relationship between them and the rate of volatile production.

A brief review of the methods employed to measure organic vapors arising from fruits has been given by Gerhardt and Ezell (*11*).

Combustion techniques as employed by Gane (*7, 9*) and Kidd and West (*14*) offer one of the most suitable laboratory methods of removing all the organic materials arising from fruits if the rate of air flow is slow.

Concentrated sulfuric acid (specific gravity, 1.84) was suggested by Gerhardt and Ezell (*11*) as a suitable oxidizing agent for all the known organic emanations arising from apples. Later, Gerhardt (10) recognized that concentrated sulfuric acid was ineffective as an ethylene absorbent in his previous experiments. However, Tropsch and Mattox (*25*) found that sulfuric acid treated with nickel and silver sulfates was able to remove ethylene from gaseous hydrocarbons much more efficiently than untreated acid. Consequently, Gerhardt (*10*) assumed that ethylene was absorbed from a stream of air containing apple emanations when the vapors were passed through 10 ml. of an activated acid mixture (1 part concentrated sulfuric acid saturated with nickel sulfate at room temperature to 15 parts of acid possessing 0.6 percent of silver sulfate at room temperature).

Walls (*26*), using an air flow of 3 liters per hour, found that 90 percent of the ethylene was removed from air containing 1 part of ethylene per 10,000 of air, if 0.8 gm. of silver sulfate was added to 40 ml. of concentrated sulfuric acid. He found silica gel and activated carbon (Desorex III) ineffective at room temperature in adsorbing ethylene. The materials were suitable, however, when cooled to minus 40° C.

Smith and Gane (*18*) were able to remove the ripening agent (ethylene) from apple vapors by the use of bromine, ozone, fuming nitric, and fuming sulfuric acid.

It is obvious that those materials which are most effective in removing apple vapors from the atmosphere are unsuitable for commercial use, with the possible exception of ozone. The evidence obtained from the use of ozone either as a scald control measure (*3*) or as an oxidizing agent for apple emanations (*18*) is inconclusive at this time.

METHODS

The experiments were carried out in 1942 with apples collected from the Cornell University orchard at Ithaca, N. Y. Blemish-free fruits were selected, and composited, and placed in 18-liter jars. Special precautions were taken so that none of the experimental apples were exposed to vapors from ripe apples prior to the experimental period.

The jars containing the fruit were placed in a constant temperature water bath where the temperature was maintained at 74° F., unless otherwise stated. A mercury pump was employed as a means of supplying air movement around the fruit. The air passing over the apples was brought to that of the bath by first being drawn through copper coils immersed in the bath. The absorption period of volatile emanations from the apples generally lasted 20 hours. The respiration measurements were made during the subsequent 4-hour period on the same lot of fruit.

PROCEDURES EMPLOYED FOR MEASURING ORGANIC APPLE EMANATIONS
AND OIL VAPORS

Absorption by sulfuric acid.—The measurements of organic apple, oil, and ethyl acetate vapors were made by using a method similar to one described by Gerhardt and Ezell (*11*). This method was modified somewhat by employing higher temperatures when oxidizing the vapors absorbed in concentrated sulfuric acid (specific gravity, 1.84). The reasons for this modification have already been discussed (*23*). Briefly, this procedure consists of absorbing apple emanations, from a stream of air, in towers containing 35 ml. of concentrated sulfuric acid and then oxidizing them with known volumes of a standard solution of ceric sulfate (approximately 0.1 N) in at least a 1 molar concentration of acid. The oxidation process was carried out at 15 pounds' steam pressure (approximately 121° C.) for at least 6 hours. The excess ceric sulfate was titrated with 0.1 N ferrous sulfate; orthophenanthroline ferrous sulfate was used as the indicator (*19*). The results are expressed as milligrams of ceric sulfate reduced per kilogram of fruit per 20 or 24 hours. This method is not suitable for ethylene absorption, however, since ethylene is not readily absorbed in concentrated sulfuric acid (*10*).

Absorption by sodium bisulfite.—Aldehyde determinations were made by Tomoda's method (*24*). The measurements were obtained by placing towers containing 40 ml. of a solution of concentrated sodium bisulfite in series with the tower containing the concentrated sulfuric acid. These towers were so arranged that the apple vapors originally passed through the sodium bisulfite and, consequently, only aldehyde-free vapors reached the tower containing the sulfuric acid. Since acetaldehyde is a known constituent of apple vapors (*16*), the aldehydes were calculated as milligrams of acetaldehyde per kilogram of fruit per 20 or 24 hours. Further details concerning the method have appeared elsewhere (*23*).

MATERIALS EMPLOYED FOR ABSORBING ETHYL ACETATE AND APPLE VAPORS

Oils.—An attempt was made to determine how effective certain oils (products of the Shell Company) would be in absorbing the ester, ethyl acetate, since it has been thought by some (*3*) that esters are related to the production of apple scald. These determinations were made by passing known weights of ethyl acetate vapors through 25-ml. samples of oil at the rate of 28 to 36 liters per hour. Any ethyl acetate which was not removed by the oils was taken up by a tower of sulfuric acid and the milligrams of ceric sulfate reduced were calculated. The apparatus used has been described in a previous paper (*23*).

Further determinations were made with oils to ascertain their capacity for removing apple emanations. In these experiments lots of McIntosh apples were placed in a temperature-control bath at 74° F. The quantity of organic material arising from the fruit was determined for a few days before samples of the oils were inserted in the series between the fruit and the concentrated acid. By following this procedure it was hoped that the percent of the total apple vapors absorbed by the oils could be determined.

Activated charcoal.—Since activated charcoals are frequently used for adsorbing organic gases and impurities from liquids, a number of tests were made with an activated lignite charcoal (Darco) on the re-

moval of ethyl acetate and organic apple emanations. Samples of both powdered and granular (4 by 12 mesh) were employed. The ability of the charcoal to adsorb ethyl acetate and organic apple vapors was determined at room temperature by following procedures similar to those outlined under the discussion of methods of testing oils. Other details relating to the use of charcoal are presented with the results.

MATERIALS EMPLOYED FOR REMOVING ETHYLENE FROM APPLE VAPORS

Kidd and West (*13*) and Smock (*20*) have shown that ripe apple vapors will stimulate the respiratory rate of preclimacteric apples. Since ethylene has a similar effect on immature apples it has been assumed that ripe apples liberate sufficient ethylene to hasten the rate of ripening of immature fruit. Therefore, the adsorbents or absorbents tested to remove this ripening agent were placed in series between post- and pre-climacteric fruit. The preclimacteric apples were held at 74° F., unless otherwise stated, whereas the postclimacteric fruit and the materials to be tested were kept at room temperature. Air was drawn at the rate of 28 liters per hour from six ripened apples over half-bushel lots of preclimacteric fruit for 2 days as indicated on the figures. Vapors from six apples of the same variety and maturity were drawn over the control lot. Daily measurements were made of the rate of respiration and volatile production of the test fruit. By observing the changes in the rate of respiration, volatile production, and firmness of flesh one can determine which materials have removed ethylene from the ripe apple vapors. At least it will be possible to tell which materials have eliminated ehtylene to a point where it no longer hastens ripening of immature apples.

Oils.—The oils used were obtained from the Sun Oil Co. Fifty-milliliter samples of the oils were placed in towers 24 inches high and 1 inch in diameter. These columns were filled with tightly packed steel wool so that a large surface of oil would be exposed to the ripe apple vapors as they passed through the absorption towers. Oil D3 was napthenic while oils I1 and A5 were paraffinic in nature.

Activated charcoal.—Samples of granular, activated, lignite charcoal (4 by 12 mesh) were placed in towers similar to those used for the oils.

Brominated, activated charcoal.—Samples of granular, activated, lignite charcoal were saturated with bromine. These samples were then aerated in an effort to remove as much of the "free" bromine vapors from the charcoal as possible. However, special precautions, as discussed later, were necessary to remove the bromine vapors which arose from the towers.

Alkaline potassium permanganate.—The solution used consisted of 50 milliliters of water saturated with potassium hydroxide combined with 50 milliliters of water saturated with potassium permanganate. The absorption tower employed was similar to the one used for the oils.

Activated sulfuric acid.—Thirty-five milliliters of this material were prepared as directed by Tropsch and Mattox (*25*). The solution consists of 1 part concentrated sulfuric acid (specific gravity, 1.84) saturated with nickel-sulfate at room temperature to 15 parts of acid possessing 0.6 percent of silver sulfate. The solution was placed in an absorption tower containing solid glass beads.

RESULTS·

MEASUREMENTS OF THE VOLATILE PRODUCTS LIBERATED BY SOME OILS

Before the value of the oils as absorbents of some organic emanations could be determined, it was necessary to find the quantity of volatile material released by the oils themselves.

The results are given in table 1. Spuria 22 and Latus 29 were oils of low refinement, Aurita 22 was of medium refinement, and Oliva 34 was of high refinement. Oliva 34 was also a nonoxidizing oil. There is a definite correlation between the refinement of an oil and the total volatile material produced. Oils of low refinement produce the greatest amount of volatile substances. The refinement of the Protego oil was unknown. In general, less volatile material was released by these oils with time.

TABLE 1.—*Volatiles liberated from 25-ml. samples of different oils*

Oil type	Ceric sulfate reduced per day by sample No.—					Total
	1	2	3	4	5	
	Mg.	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
Spuria 22	207.8	424.3	292.3	150.7	135.6	1,210.7
Latus 29	57.9	101.9	92.0	89.2	59.5	400.5
Aurita 22	60.7	71.8	61.9	35.7	40.8	270.9
Oliva 34	38.5	35.7	49.2	30.1	35.2	188.7
Protego	104.6	90.7	73.0	13.9	35.2	317.4
Air	25.0	34.5	31.8	8.4	8.4	108.1

ABILITY OF CERTAIN OILS AND ACTIVATED CHARCOAL TO REMOVE VAPORS OF ETHYL ACETATE AND SOME APPLE EMANATIONS

OILS

The effectiveness of oils in absorbing vapors of ethyl acetate was determined by passing known weights of ethyl acetate vapors through 25-ml. samples of oil.[4] Any ethyl acetate which was not removed by the oil was taken up by a tower of sulfuric acid and the milligrams of ceric sulfate reduced were calculated. Since the oils themselves liberate volatile material, it was realized that some of the ceric sulfate was reduced by the emanations from the oils as well as from the ethyl acetate which passed unabsorbed through the oil. However, these measurements lasted only 1 hour per day so that the amount of volatile material arising from the most unrefined oils probably did not exceed 20 mg. of ceric sulfate reduced. From the results shown in table 2, where quantities from 845 to 3,500 mg. of ceric sulfate were reduced, it can be seen that even 20 mg. of ceric sulfate reduced by the oil volatiles would be insignificant. The reduction-oxidation ratios,[5] the milligrams of ethyl acetate absorbed, and the percent absorption, were calculated. It was assumed that all the ceric sulfate reduced was due to the ethyl acetate alone. The milligrams of ethyl acetate absorbed and the percent absorption were calculated assuming the average reduction-oxidation value to be 28.27 as shown by Southwick (*23*).

[4] The apparatus used has been described (*23*).
[5] A term employed by Gerhardt and Ezell (*11*)

TABLE 2.—*The absorptive capacity of some oils for ethyl acetate*

Oil type (25 ml.)	Ethyl acetate	Ceric sulfate reduced	Reduction-oxidation ratio	Ethyl acetate absorbed	Degree of absorption
	Milligrams	*Milligrams*		*Milligrams*	*Percent*
First day:					
Spuria 22	87.9	1,517.2	17.26	34.2	38.9
Latus 29	61.7	845.8	13.71	31.8	51.5
Aurita 22	76.9	1,433.2	18.64	26.2	34.1
Aurita 32	84.0	1,618.7	19.27	26.7	31.8
Oliva 27	74.3	1,529.0	20.58	20.2	27.2
Oliva 34	79.8	1,484.4	18.60	27.3	34.2
Protego	79.1	1,592.4	20.13	22.8	28.8
Second day:					
Spuria 22	99.6	2,828.9	28.40	−.5	−.5
Latus 29	79.4	1,942.9	24.47	10.7	13.5
Aurita 22	78.2	2,174.5	27.81	1.3	1.7
Aurita 32	71.3	1,903.9	26.70	4.0	5.6
Oliva 27	65.5	1,788.8	27.31	2.2	3.4
Oliva 34	91.9	2,638.7	28.71	−1.4	−1.5
Protego	153.6	3,540.2	23.05	28.4	18.5

One difficulty in making direct comparisons of one oil with another is that the quantities of ethyl acetate passing through each oil were not identical. The data do indicate that the most unrefined oils, Spuria 22, Latus 29, and Protego, are somewhat superior to the highly refined oils so far as their capacities for absorbing ethyl acetate is concerned. None of the oils appear to be very effective, however. It is suggested that the reason the sample of Protego seems so much superior to the other oils on the second day may be due to the larger concentration of ethyl acetate passing through it during the absorption period. A higher vapor pressure of ethyl acetate in this case may well have resulted in a greater absorption of this ester in the oil.

Further determinations were made with a number of oils. In these experiments lots of McIntosh apples were placed in a temperature control bath at 74° F. The quantity of organic material absorbed in surfuric acid and the quantity of ceric sulfate reduced were recorded by the usual procedure. Then towers containing 25-ml. samples of oil were inserted in the series between the fruit and the concentrated acid. It is apparent that the oils removed some of the volatile material since the amount of ceric sulfate reduced was higher before and after the oils were removed from the series (table 3). Although the apples were held at a constant temperature of 74° F., the rate of volatile production was not constant from day to day. Consequently, the actual amount of volatile material removed by the oils could not be calculated. It was though tthat perhaps the quantity of material absorbed by the oils could be measured by aerating them after they had been subjected to apple emanations. This was done on January 19 and January 20 as indicated in table 3. The results show that there was more volatile material arising from these oils after the first day of aeration than after the second day. Since the quantities of organic vapors emitted from the oils themselves, as shown in table 1, were not constant, it cannot be assumed that the difference in volatile production from January 19 to January 20 (table 3) represents only the apple emanations which had been removed from the oils. However, by comparing the loss in organic vapors from oils which had been aerated for 5 days (table 1) with the losses of the same oils in this experiment after an equal number of days, one can see that the amounts of ceric sulfate reduced are quite similar.

TABLE 3.—*The ability of oils to absorb vapors from 10 McIntosh apples at 74° F*

Date	Treatment	Ceric sulfate reduced per day by—					
		Aurita 22	Latus 29	Spuria 22	Oliva 34	Protego	No oil
		Mg.	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
Jan. 11	Apples alone	648.3	632.6	650.7	688.8	512.7	680.8
12	....do....	493.2	672.3	389.3	697.8	389.3	624.1
13	....do....	498.4	334.4	585.2	732.1	301.0	722.4
14	....do....	739.2	692.7	548.4	712.5	444.9	528.6
15	Oils added	481.8	458.7	488.5	651.5	308.8	541.6
16	....do....	463.5	476.6	403.2	546.8	306.9	503.5
17	....do....	435.8	452.4	409.6	521.8	297.4	549.1
18	Apples alone	680.4	703.4	561.4	749.4	459.4	759.3
19	Oils only	213.3	115.0	269.7	154.2	49.2	[1] 13.1
20	....do....	42.4	58.6	111.1	26.2	26.2	[1] 9.9

[1] Air.

ACTIVATED CHARCOAL

The adsorptive capacity of 25 gm. of powdered, activated lignite charcoal (Darco) for ethyl acetate was measured by employing the same procedure and air flow as was used with the oils, except that the length of time per treatment lasted from 1 to 8 hours. The data are presented in table 4. A comparison of the data in the last column of tables 2 and 4 shows the charcoal to be vastly superior to the oils insofar as its capacity to remove this ester is concerned. The charcoal was practically a perfect adsorbent for the first 1.8 gm. of ethyl acetate which passed through it. The adsorptive capacity of the charcoal dropped markedly during the last treatment after approximately 2.6 gm. had been absorbed.

In an effort to determine how effective this activated charcoal would be in adsorbing the volatile materials arising from apples which react with sulfuric acid, an 80 gm. sample of charcoal was tested. This sample of granular, activated charcoal was selected because it had been used previously to absorb apple volatiles for 5 months from a bushel of Cortland and a bushel of McIntosh apples held at 40° F. The author was anxious to know whether this sample of charcoal had become saturated with adsorbed apple vapors during the 5-month period. The emanations from 10 ripe Cortland apples at room temperature were absorbed in sulfuric acid and the quantity of material produced on April 4 and 5 was determined in terms of milligrams of the sulfate reduced. On April 6 and 7 the charcoal was inserted, as an adsorbent, in series between the apples and on April 8 and 9 the concentrated acid was removed. The results are given in figure 1.

TABLE 4.—*The adsorptive capacity of 25 gm. of powdered activated charcoal for ethyl acetate*

Treatment No.	Ethyl acetate	Ceric sulfate reduced	Reduction-oxidation ratio	Ethyl acetate adsorbed	Degree of adsorption
	Milligrams	*Milligrams*		*Milligrams*	*Percent*
1	88.4	45.8	0.52	86.8	98.19
2	228.7	27.5	.12	227.7	99.56
3	627.8	7.6	.01	627.5	99.95
4	861.0	6.4	.01	860.8	99.98
5	796.9	5,099.5	6.40	616.5	77.36
6	79.2	1,872.5	23.64	13.0	16.41

This same sample of charcoal was also subjected to vapors from one-half bushel of ripe McIntosh apples on April 8 and 9. The daily rate of volatile production of the McIntosh apples was determined for 3 days (April 5, 6, and 7) before the activated charcoal was tested. On April 10 no material was used in removing apple vapors, but on April 11 a 25-ml. sample of fresh Protego oil was employed as an absorbent. No adsorbent was used on April 12. The comparative abilities of the activated charcoal and Protego oil
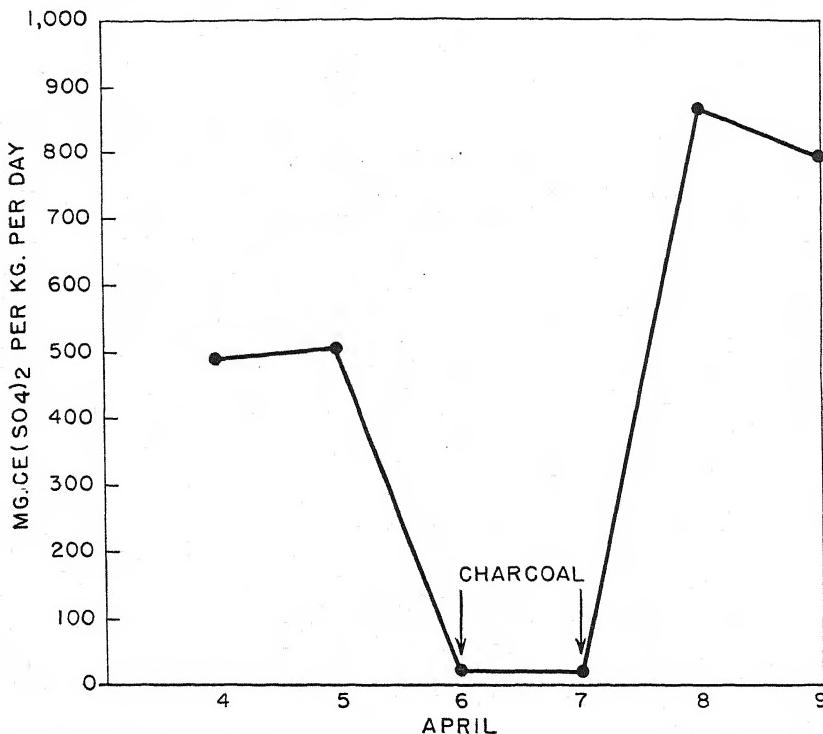
FIGURE 1.—Adsorptive capacity of activated charcoal for apple volatiles that react with concentrated sulfuric acid, as indicated by experiments conducted on Cortland apples at room temperature. Arrows indicate when charcoal was used to adsorb apple emanations.

to remove aldehydes and organic apple emanations is shown in figure 2. It is apparent that granular, activated lignite charcoal is an excellent adsorbent for apple vapors that react with concentrated sulfuric acid and that Protego oil is very much inferior to it. It is also apparent that the charcoal was not saturated with the apple vapor arising from apples held for 5 months at 40° F.

VALUE OF DIFFERENT MATERIALS FOR REMOVING THE RIPENING AGENT (ETHYLENE) FROM APPLE EMANATIONS

Although the results shown in figures 1 and 2 demonstrate that activated lignite charcoal is effective in removing apple vapors that react with concentrated sulfuric acid, the problem of determining which materials remove ethylene has yet to be solved.

In the experiments described below the value of several oils, activated charcoal, brominated, activated charcoal, alkaline potassium permanganate, and activated sulfuric acid as ethylene removers was determined. Preclimacteric Wealthy, McIntosh, and Rhode Island Greening apples were selected and held at 74° F. (except in the experiment noted in figure 3, where the temperature was maintained at 78° F.). Vapors from six ripened apples were passed over half-bushel lots of unripe fruit for 2 days, as indicated by arrows in figures 3, 4,
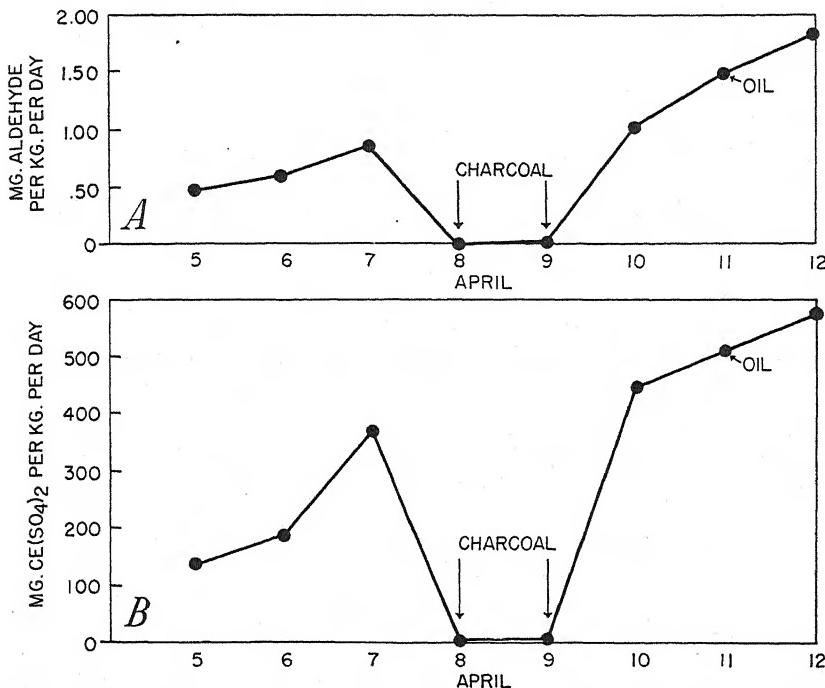
FIGURE 2.—Adsorptive and absorptive capacity of activated charcoal and oil, respectively, for apple volatiles that react with concentrated sulfuric acid, as shown by experiments conducted at room temperature on: *A*, Cortland apples; *B*, McIntosh apples. Arrows indicate when charcoal and oil were used to remove apple emanations.

5, 6, and 7. Vapors from six apples of the same variety and stage of maturity were drawn over the control lot at the same time.

In the first of this series of experiments (figs. 3) three oils (products of the Sun Oil Co.) were studied. None of the 50-ml. samples of these oils was capable of lowering the concentration of the ripening agent contained in ripe Yellow Transparent apple vapors below its stimulatory level. This conclusion is substantiated by the volatile measurements as well as by the respiratory determinations. The control lot was also firmer than the other groups of fruit. However, the apples which had been subjected to Yellow Transparent apple emanations, in the absence of oil as an absorbent, were softest.

Figure 4 shows the results of an experiment where 100 ml. of alkaline potassium permanganate, 80 gm. of granular, activated charcoal, and
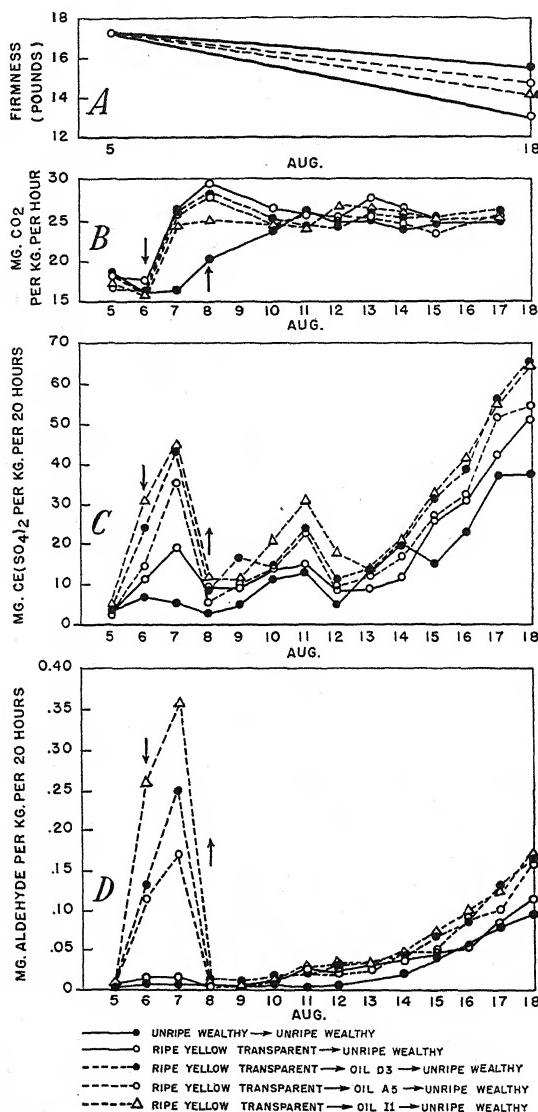
FIGURE 3.—A comparison of the firmness, respiration, and volatile production of unripe Wealthy apples treated with vapors from ripe Yellow Transparent at a temperature of 78° F., and of the ability of three commercial oils to remove a ripening agent (ethylene) from these vapors. *A*, Firmness; *B*, carbon dioxide given off per kilogram per hour; *C*, ceric sulfate reduced per kilogram in 20 hours; *D*, aldehyde produced per kilogram in 20 hours. Downward-pointing arrows indicate start of 2-day treatment with apple vapors; upward-pointing arrows, the end of this treatment.
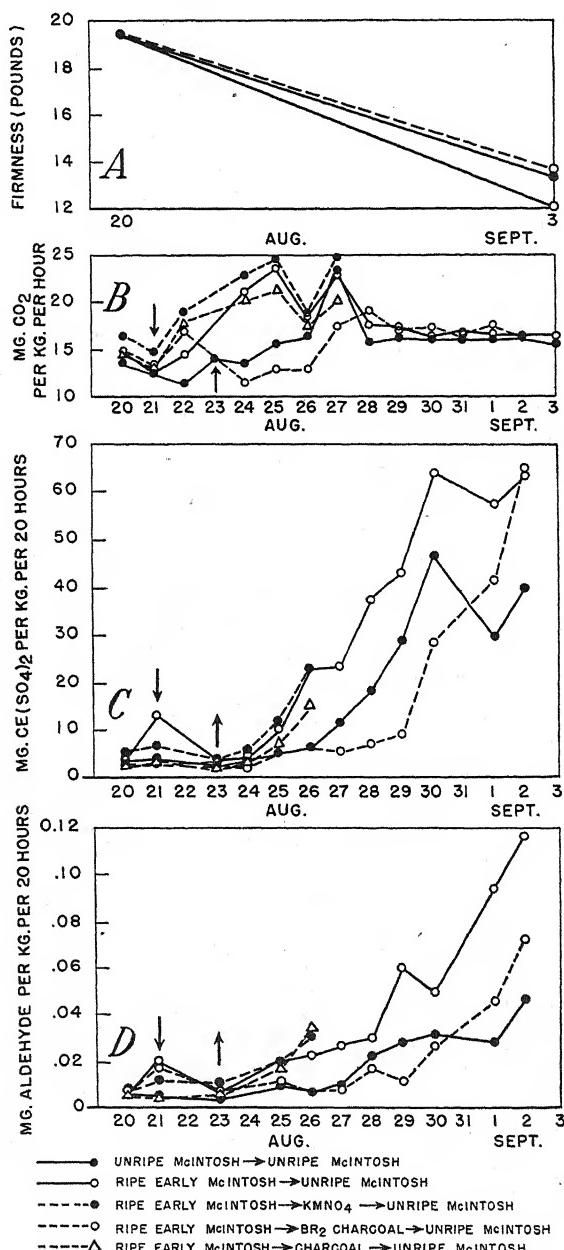
FIGURE 4.—A comparison of the firmness, respiration, and volatile production of unripe McIntosh apples, treated with vapors from ripe Early McIntosh apples at a temperature of 74° F., and of the ability of alkaline potassium permanganate, activated charcoal, and brominated, activated charcoal to remove a ripening agent (ethylene) from these vapors. *A*, Firmness; *B*, carbon dioxide given off per kilogram per hour; *C*, ceric sulfate reduced per kilogram in 20 hours; *D*, aldehyde produced per kilogram in 20 hours. Downward-pointing arrows indicate start of 2-day treatment with apple vapors; upward-pointing arrows, the end of this treatment.
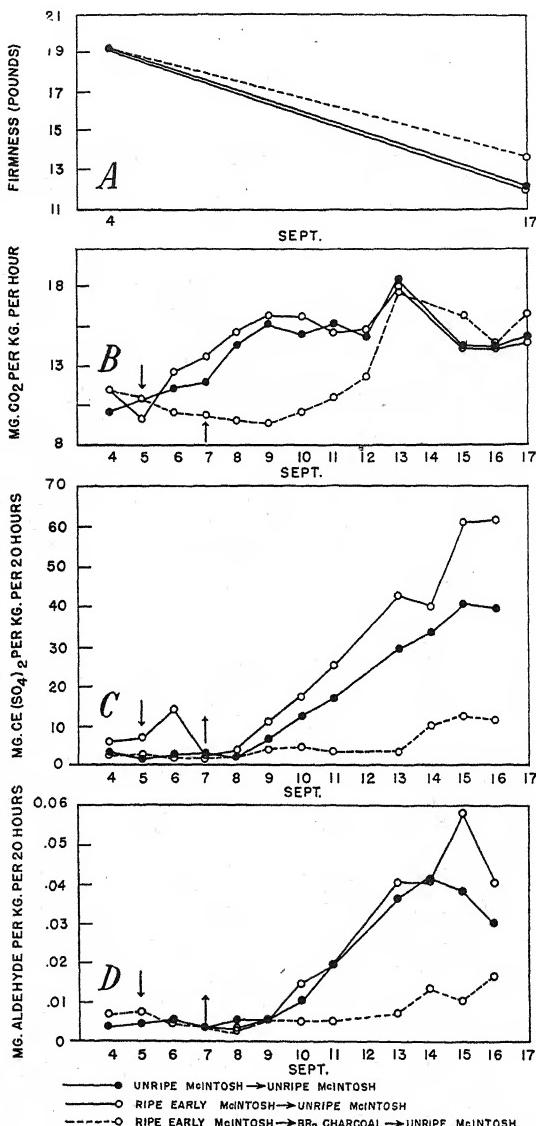
FIGURE 5.—A comparison of the firmness, respiration, and volatile production of unripe McIntosh apples treated with vapors from ripe early McIntosh apples at a temperature of 74° F., and of the ability of brominated, activated charcoal to remove a ripening agent (ethylene) from these vapors. *A*, Firmness; *B*, carbon dioxide given off per kilogram per hour; *C*, ceric sulfate reduced per kilogram in 20 hours; *D*, aldehyde produced per kilogram in 20 hours. Downward-pointing arrows indicate start of 2-day treatment with apple vapors; upward-pointing arrows, the end of this treatment.

the same weight and type of charcoal saturated with bromine were used to remove ethylene from ripe early McIntosh apple emanations.

In all instances except those in which the brominated charcoal was employed the ripe apple emanations stimulated an early climacteric rise in respiration and volatile production as compared to the check. However, some of the bromine from the brominated charcoal passed over this experimental lot of apples. This was evident from the fact that a solution of sodium hydroxide placed in the series for a few hours became light yellow as the bromine vapors reacted with it. As a consequence, one cannot be certain whether the bromine held on the charcoal or the bromine in the air stream was responsible for the removal of the active agent in the Early McIntosh vapors. The apples in the group in which the brominated charcoal was employed were as firm as the control lot at the end of the experiment.

The results recorded in figure 5 substantiate those shown in figure 4. In this experiment the presence of brominated charcoal appeared to have retarded the initiation of the climacteric rise in respiration and volatile production. The control lot produced volatiles and carbon dioxide at a rate comparable to that of the apples treated with ripe apple vapors in the absence of an adsorbent. The marked delay in the development of the climacteric rise in respiration and volatile production of those McIntosh apples with which brominated charcoal was used may have been due to the presence of bromine on the surface of the apples. In this experiment, as in the previous one (fig. 4), some bromine vapor was carried over to the experimental fruit in the air stream even though a sodium hydroxide gas washing solution was employed. The presence of free bromine on the surface of these apples might have reacted with any ethylene produced by the apples themselves, and so have delayed the climacteric rise in respiration and volatile production.

An effort was made in the experiment presented in figure 6 to determine whether the ability of brominated charcoal to remove the active agent in ripe apple vapors was due to the bromine vapors arising from the brominated charcoal or to the bromine actually adsorbed on the charcoal. Forty grams of the saturated bromine charcoal were mixed with an equal part of unbrominated, activated charcoal. By such a procedure any excesses of loosely held bromine were adsorbed by the unbrominated fraction. After this mixture was prepared and well stirred no bromine could be detected by the sense of smell or by the appearance of a light yellow color when air from this charcoal was passed through a solution of sodium hydroxide. This 80-gm. sample was placed in a tower between the six ripe McIntosh and the half bushel of preclimacteric Rhode Island Greening apples. A 40-gm. sample of the saturated bromine charcoal was placed before the ripe McIntosh apples, as shown in figure 6, so that the bromine vapors arising from this charcoal passed over the ripe fruit as well as over the preclimacteric Rhode Island Greening apples.

The results show that brominated charcoal may remove ethylene from ripe apple vapors even in the absence of "free" bromine vapors.

An attempt was made to determine whether 35 ml. of activated sulfuric acid, prepared as directed by Tropsch and Mattox (25), would remove ethylene from apple vapors below a stimulatory concentration. Vapors from ripe Rhode Island Greening apples were
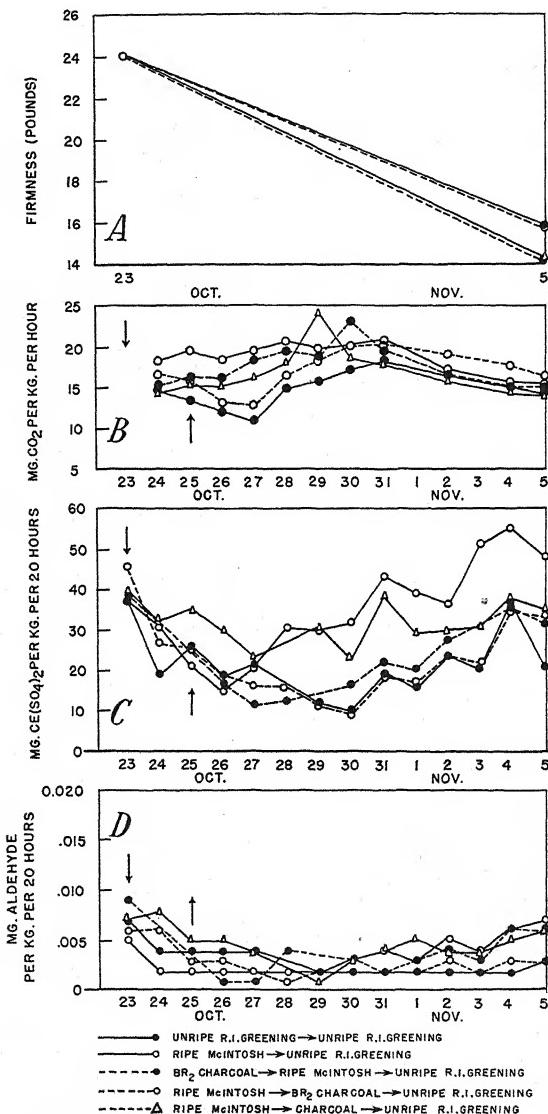
FIGURE 6.—A comparison of the firmness, respiration, and volatile production of unripe Rhode Island Greening apples treated with vapors from ripe McIntosh apples at a temperature of 74° F., and of the ability of activated charcoal, brominated, activated charcoal, and the vapors from brominated activated charcoal to remove a ripening agent (ethylene) from these vapors. *A*, Firmness; *B*, carbon dioxide given off per kilogram per hour; *C*, ceric sulfate reduced per kilogram in 20 hours; *D*, aldehyde produced per kilogram in 20 hours. Downward-pointing arrows indicate start of 2-day treatment with apple vapors; upward-pointing arrows, the end of this treatment.

drawn over preclimacteric apples of the same variety after these ripe apple emanations had passed through the adsorption tower containing the activated acid. The results shown in figure 7 indicate that the
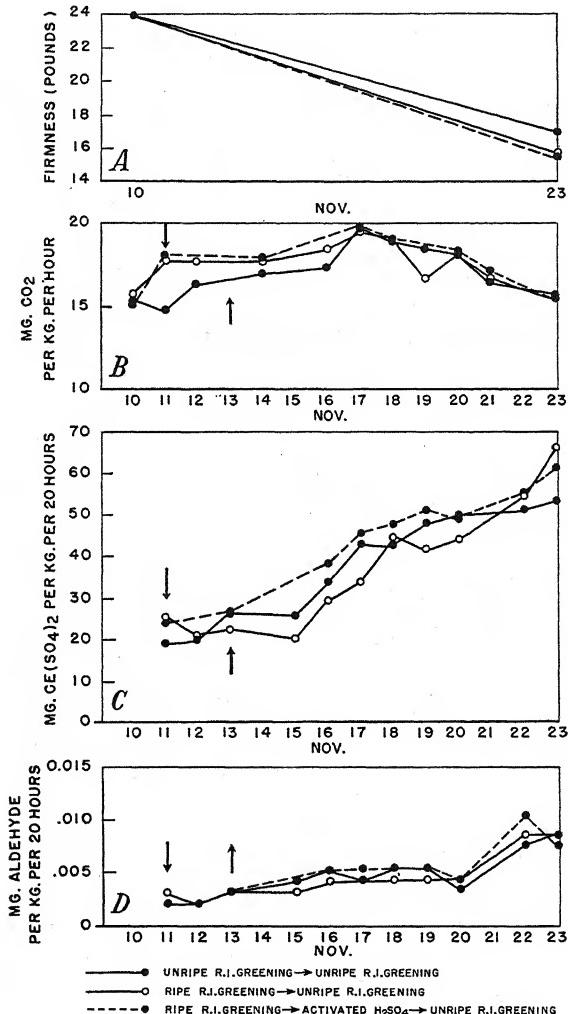
FIGURE 7.—A comparison of the firmness, respiration, and volatile production of unripe Rhode Island Greening apples treated with vapors from ripe Rhode Island Greening apples at 74° F., and the ability of activated sulfuric acid to remove a ripening agent (ethylene) from these vapors. *A*, Firmness; *B*, carbon dioxide given off per kilogram per hour; *C*, ceric sulfate reduced per kilogram in 20 hours; *D*, aldehyde produced per kilogram in 20 hours. Downward-pointing arrows indicate start of 2-day treatment with apple vapors; upward-pointing arrows, the end of this treatment.

ethylene in the vapors from the ripe Rhode Island Greening apples was not removed below the level of respiratory stimulation when an air flow of 28 liters per hour was employed. The inability of the activated sulfuric acid to absorb the ethylene from the vapors arising

from the postclimacteric fruits in this experiment may have been due to the rapid rate of air flow used.

DISCUSSION

The results of this study indicate that of all the materials tested, brominated, activated charcoal is the one which most nearly approaches the ideal atmospheric washing agent from the standpoint of its ability to remove all the known organic apple emanations. Activated charcoal and the oils tested appear to be ineffective in removing ethylene from the atmosphere surrounding stored apples. Moreover, the oils were very inferior to the activated charcoal in removing the apple emanations that react with concentrated sulfuric acid.

From the evidence presented, it seems reasonable to expect that brominated, activated charcoal would be the most suitable of the materials tested for delaying ripening in storage, but, since the gas or combination of gases liberated by apples which influence the development of scald is not definitely known, it is virtually impossible to predict which, if any, of the materials tested would be suitable for scald control. On the basis of their ability to remove all apple emanations except ethylene, one might expect the oils to be inferior to activated charcoal in controlling scald. Brominated, activated charcoal might prove to be superior to activated charcoal if ethylene is related to the incidence of scald.

No commercial trials have been conducted to ascertain the value of oils, of activated charcoal, or of brominated, activated charcoal as a means of delaying ripening or controlling scald in commercial storage. The value of these materials may depend on the rate and completeness of air circulation within a storage room as well as upon the chemical and physical properties of the substance employed as the atmospheric washing agent. If one is correct in assuming that apple emanations are related to the appearance and severity of scald as well as to the ripening of immature apples, one could hardly expect any materia used to "air-condition" a storage room to be satisfactory unless the organic gases liberated from the fruits are rapidly removed from the atmosphere surrounding the fruits.

SUMMARY

The primary object of this study was to find a suitable material that would react with all the known organic vapors arising from apples.

Activated charcoal was distinctly superior to all the oils tested in its ability to remove vapors of ethyl acetate and the organic apple emanations which react with concentrated sulfuric acid.

The oils, activated charcoal, alkaline potassium permanganate, and activated sulfuric acid were unable to remove the ethylene from postclimacteric apple vapors. At least sufficient quantities of ethylene remained in the air stream after passing through these materials to hasten the appearance of the respiratory and volatile climacteric of immature apples.

Activated charcoal impregnated with bromine was sufficiently reactive at room temperatures to remove ethylene from the vapors arising from ripe apples. The rate of respiration and volatile produc-

tion of preclimacteric apples was not stimulated by the ethylene in ripe apple vapors after the vapors had passed through the brominated, activated charcoal.

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A BIOCHEMICAL STUDY OF SOIL ORGANIC MATTER AS RELATED TO BROWN ROOT ROT OF TOBACCO [1]

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INTRODUCTION

Within the past few years it has become generally recognized that certain crops grown in rotation may have an undesirable effect upon the succeeding crop. Such an effect has frequently been observed on tobacco (*Nicotiana tabacum* L.), and in its most severe form when tobacco has been preceded by a sod crop, particularly timothy. The roots of tobacco growing under these conditions often become discolored or decayed. This condition has led to the use of the term "brown root rot." Previous investigations (5)[2] have demonstrated that an organism, *Rhizoctonia bataticola* (Taub.) Butler, is associated with and is a primary cause of brown root rot of tobacco. The present investigation was undertaken to determine whether or not any correlation exists between the occurrence and severity of brown root rot and the biochemical nature of the crop residues which appear to aggravate the disease.

EXPERIMENTAL PROCEDURE

The most obvious means by which a crop could influence a succeeding one would be by the residues of the first crop left in the soil. Such residues were examined biochemically, chiefly by a system of proximate analyses (7). The organic fractions investigated included that soluble in benzene-alcohol, the carbohydrate portion, various organic nitrogeneous complexes, and that portion containing lignin, herein designated as lignin-humus. The chemical distribution of soil nitrogen was investigated in a similar manner. The nitrogen fractions included nitrate nitrogen, other water-soluble nitrogen, ammonia nitrogen, amide nitrogen representing acid amides, nitrogen representing amino acids and various heterocyclic compounds, and the nitrogen of organic complexes resistant to prolonged acid hydrolysis. The results were analyzed and interpreted statistically in conjunction with measurements of the severity of brown root rot as it occurred in the soil of the various plots.

ARRANGEMENT AND TREATMENT OF PLOTS

The experimental tobacco plots from which the soils were taken were located at the Tobacco Experiment Station at Upper Marlboro, Md. Since the arrangement of plots, the rotations, fertilizer practices, and yield trends have been given in detail for the most part

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by Garner, Lunn, and Brown (*2*) only a brief description is given here. The soil was mapped as Collington. Most of it was some phase of fine sandy loam, although a few very small areas of loamy sand were found on some plots. The plots chiefly concerned in this paper were the tobacco plots of field IV and field V. The plot arrangement was identical in each field, but field IV and field V bore tobacco in alternate years. Thus field IV was sampled in 1932 and field V in 1933.

These 2 fields were divided into 2 sections of 21 plots each. Each group of 21 plots was divided into 3 groups of 7 plots each. A 2-year rotation of tobacco and grain with different cover crops was used on all plots, except the center plot of each subsection. This plot had a rotation of corn and small grain with no cover crop or fertilizer. The grain crops of the 3 subsections were respectively wheat, oats, and rye. As cover crops, vetch, crimson clover, fallow (kept free of grass and weeds), fallow (corn and small grain plot), cowpeas, soybeans, and grass were used in that order. The grass cover crop was a mixture of timothy, tall meadow oat, orchard, and Italian rye grasses. All except the grasses, which were seeded with the grain crop, were planted after the grain crop and all were plowed under the following spring before tobacco was planted. All crops except corn were fertilized with 60 pounds per acre of P_2O_5 from precipitated dicalcic phosphate and 30 pounds per acre of K_2O from sulphate of potash. Two-thirds of this was applied to tobacco and one-third to the grain crop. None of the plots received nitrogenous fertilizers.

METHODS OF SAMPLING AND ANALYSIS

All plots in field IV were sampled on June 21, 1932, shortly after tobacco had been planted, and were resampled on July 19 and August 23. The corresponding plots in field V were sampled in 1933 on June 18, July 19, and September 17. Because of weather conditions sampling was omitted in August 1933 and a sampling in September was substituted.

Each sample was composited from 20 individual cores of soil. Each core was taken about 10 inches from the plant and about 7 inches from the center of the row. All composite samples were dried at 60° to 65° C., passed through an 8-mesh sieve to remove gravel and the coarser plant material, and then thoroughly mixed.

Nitrate, water-soluble, and ammonia nitrogen were determined on the samples thus prepared. For all other determinations, samples were ground to 40–60 mesh in a Braun pulverizer in order to obtain a more uniform distribution of the organic material in the soil. Nitrate nitrogen was determined by Harper's modification of the phenoldisulfonic acid method (*3*), using a 1 to 5 dilution of the soil. Ammonia nitrogen was estimated in 50-gm. samples by replacement with sodium carbonate according to the aeration method of Matthews (*4*). For water-soluble nitrogen, 100 gm. of soil were shaken at intervals for 30 minutes with 250 ml. of distilled water, filtered through Whatman No. 12 paper, and two 100-ml. aliquots of the filtrate taken for analysis. The Kjeldahl method modified to include nitrate nitrogen was used.

Total nitrogen and total carbon were determined on the pulverized soil. The Kjeldahl method modified to include nitrates was used for total nitrogen. Total carbon was determined by wet combustion, the carbon dioxide being estimated by absorption in standard alkali and

titration of the excess alkali with standard acid.   The carbon content of the soil, multiplied by the factor 1.724, was taken as a measure of the total organic matter (7).

Further fractionation of nitrogen and organic matter was carried out according to a modification of the proximate system of Waksman and Stevens (7), as follows: 100-gm. samples of the pulverized soil were extracted overnight with 1:1 benzene-95 percent alcohol in Soxhlets. The extracts were evaporated and organic carbon of the residues determined by wet combustion.   Using the factor 1.724, this fraction represented the fatty, waxy, and resinous materials in the soil organic matter.   The extracted soils were carefully dried, transferred to 800-ml. Kjeldahl flasks, and saturated with cold 80 percent sulfuric acid for 2 hours.   Fifteen volumes (525 ml.) of distilled water were then added to each flask and the contents of the flasks thoroughly shaken.   The flasks were then connected to reflux condensers. Hydrolysis was carried out for 5 hours, after which the digests were filtered through weighed papers, and the residues washed thoroughly with distilled water.

The combined filtrates and washings were made up to 1 liter volume. Reducing sugars were determined on 200-ml. aliquots.   After neutralization of aliquots with sodium hydroxide, the resultant sesquioxide precipitates were removed by filtration and washing.   The filtrates plus washings were evaporated to small volume and the excess sodium sulphate removed from solution by adding three volumes of 95 percent ethyl alcohol, filtering, and washing with 70 percent alcohol.   Alcohol was removed by successive evaporation and addition of distilled water and the solutions finally made up to volume.   During the entire process, a chlorophenol red indicator solution was used to guard against an alkaline reaction.   Reducing sugars in the prepared solutions were determined by the official permanganate method (*1, p. 192*) and the results were calculated and expressed as cellulose.

Ammonia nitrogen was determined in 100-ml. aliquots of the hydrolysate by distillation with magnesium oxide.   The ammonia was taken as a measure of the acid amide content of the soil organic matter (7).   Total nitrogen determinations on 100-ml. aliquots included not only this ammonia (amide) nitrogen, but also all of the nitrogen rendered soluble by sulphuric acid hydrolysis.   The nonamide (total hydrolyzed minus amide) nitrogen represented various amino acids and heterocyclic compounds (7) which make up certain organic nitrogenous complexes.

Following the assumption of Waksman and Stevens (7) the "protein" or organic nitrogenous complexes of the soil organic matter were calculated by multiplying the percentages of nitrogen thus hydrolyzed by the factor 6.25 and dividing the result by the percent organic matter of the original soil, which was obtained by multiplying the percent carbon by the equally arbitrary factor 1.724.   No discussion of the assumed factors will be made here, the subject having been considered in detail elsewhere (7).

Residues from hydrolysis of the 100-gm. samples of soil were carefully dried and weighed and total carbon determined on aliquot portions. Again using the factor 1.724 the residual organic matter was calculated. Aliquots also were analyzed for total nitrogen and the results (expressed on the basis of the original soil) multiplied by 6.25.   This was

considered to be a measure of the residual organic nitrogenous complex. The difference between this fraction and the residual organic matter was designated as lignin-humus, since it consisted of lignin and its transformation products and various other microbial complexes (7).

STATISTICAL CONSIDERATIONS

For statistical analysis of the effects of cropping systems on the occurrence and severity of brown root rot, the corn plots and the plots without cover crops were omitted. The severity of brown root rot was estimated by direct examination of the tobacco plants in the field, and a root rot index of arbitrarily assigned numbers was established to indicate the severity of the disease, as, trace, 1; mild, 2; moderate, 3; heavy, 4; severe, 5; and most severe, 6.

ANALYTICAL DATA

Table 1 gives the data for total organic matter in the soil, and its proximate composition as fatty-waxy, carbohydrate, nitrogenous, and lignohumic compounds. Table 2 gives the data for total soil nitrogen and its distribution by various fractions. Root rot indices, as measures of the severity of the disease, are included in each of tables 1, 2, and 3. The coefficients of correlation between these index numbers and the various fractions of organic matter and nitrogen were calculated. The values used in determining these coefficients were those used in obtaining the averages given in tables 1 and 2.

The coefficient of correlation between brown root rot and soil organic matter is -0.368 ± 0.091, which is significant. However, analysis of variance indicates that the percentage of organic matter in the soil does not vary with the cover crop to any significant extent. Thus it would appear that it is only coincidence that severe brown root rot occurred in plots containing a small amount of organic matter. This observation is confirmed by the small amount of organic matter in the fallow-rotation plots which were also very low in organic matter and upon which there was no brown root rot.

The correlation coefficients between the various fractions of organic matter and the severity of brown root rot are significant in some cases. They are invariably negative in sign, and appear upon observation to be influenced by the level of total organic matter. That is, in general, any given fraction was low or high on any given plot as the total organic matter of the plot was low or high. By using data for the organic fractions expressed as percentages of the total, this influence of the amount of organic matter was removed and different correlation coefficients were found. These are the coefficients given in table 1. Only two are barely significant: that for the portion of the organic nitrogenous complexes calculated from the nonamide hydrolyzed nitrogen, and that for the lignin-humus fraction. These are $-.213\pm0.101$ and $+0.207\pm0.101$, respectively.

TABLE 1.—*Average organic matter content of soils of tobacco rotation plots and its proximate percentage composition as carbonaceous, nitrogenous, and carbohydrate compounds*

2-year rotation	Cover crop	Root rot index	Total organic matter in soil	Benzene-alcohol soluble fraction	Carbohydrate fraction (as cellulose)	Amide nitrogen complex	Non-amide nitrogen complex	Total hydrolyzed nitrogen complexes	Nitrogen complex insoluble after acid hydrolysis	Total organic nitrogen complexes	Lignin-humus	Total organic matter accounted for
			Percent	*Percent of total*	*Percent of total*	*Percent of total*	*Percent of total*	*Percent of total*	*Percent of total*	*Percent of total*	*Percent of total*	*Percent*
Tobacco and wheat	Vetch	2.5	0.7501	5.29	6.53	8.22	15.16	23.38	15.96	39.34	41.07	92.23
Do	Clover	2.0	0.8185	5.30	7.70	10.17	15.81	25.98	17.22	43.20	41.31	97.56
Do	None	0 [1]	0.6974	4.96	6.94	7.38	14.16	21.54	15.63	37.17	41.93	91.00
Corn and wheat	do	0 [1]	0.7024	5.68	7.62	6.62	13.74	20.36	16.09	36.45	41.55	91.30
Tobacco and wheat	Cowpeas	1.5	0.8221	5.54	7.98	8.46	14.97	23.95	15.72	39.67	41.92	95.11
Do	Soybeans	1.5	0.8324	5.82	7.32	7.40	15.49	22.37	14.92	37.12	42.01	90.72
Do	Grass	4.0	0.7469	4.89	8.05	7.82	14.38	22.20	17.64	44.91	40.20	92.07
Tobacco and oats	Vetch	2.0	0.7683	4.68	7.49	9.26	18.01	27.27	17.37	42.94	40.33	97.28
Do	Clover	1.5	0.7971	5.28	6.51	8.79	16.78	25.57	17.34	41.76	41.38	97.14
Do	None	0 [1]	0.6144	5.43	7.28	8.69	15.73	24.42	15.71	39.16	45.19	95.08
Corn and oats	do	0 [1]	0.5944	5.51	7.87	8.60	14.85	23.45	17.16	41.76	41.36	97.14
Tobacco and oats	Cowpeas	1.0	0.7521	4.72	7.16	7.51	17.09	24.60	14.85	39.80	40.75	95.71
Do	Soybeans	1.0	0.7121	5.59	9.06	8.64	16.31	24.95	15.53	37.30	42.52	93.21
Do	Grass	3.5	0.6523	4.73	6.94	6.97	14.80	21.77	15.07	39.69	42.10	93.61
Tobacco and rye	Vetch	1.5	0.6803	5.46	6.51	8.78	15.84	24.62	14.56	37.39	42.91	94.19
Do	Clover		0.7276	5.22	5.61	8.24	14.59	22.83	15.34	35.98	43.76	92.03
Do	None	0 [1]	0.5476	5.72	6.97	6.44	14.20	20.64	15.34	37.36	43.48	91.07
Corn and rye	do	0 [1]	0.5076	5.17	7.35	7.50	18.69	21.19	16.17	37.36	39.43	93.08
Tobacco and rye	Cowpeas	1.5	0.6531	5.20	7.29	7.12	15.03	22.10	16.31	38.41	41.23	90.39
Do	Soybeans	1.0	0.6595	4.94	8.02	7.86	14.98	22.89	16.32	39.21	41.36	92.67
Do	Grass	4.5	0.6200	5.73		7.52	14.98	22.50	15.09	37.59	41.36	92.70
Correlation (*r*) with root rot index [2]			−0.368	+0.040	−0.003	+0.026	−0.213	−0.161	+0.020	−0.107	+0.207	
Standard error			±0.091	±0.105	±0.105	±0.105	±0.101	±0.103	±0.105	±0.104	±0.101	

[1] Brown root rot absent; these plots not included in statistical analysis.
[2] These correlations calculated from original values of which the figures in this table are averages.

TABLE 2.—*Biochemical distribution of nitrogen in the soils of tobacco rotation plots*

2-year rotation	Cover crop	Root rot index	Total nitrogen content of soil	Nitrate nitrogen fraction	Water-soluble fraction other than nitrate	Ammonia nitrogen fraction	Biochemical distribution of soil nitrogen — Sulfuric acid hydrolyzable nitrogen: Amide nitrogen fraction	Non-Amide nitrogen	Total hydrolyzable fraction	Nitrogen in organic complexes — Nitrogen fraction insoluble after acid hydrolysis	Total nitrogen in organic complexes	Total nitrogen accounted for
			Percent	*Percent of total*	*Percent of total*	*Percent of total*	*Percent of total*	*Percent of total*	*Percent of total*	*Percent of total*	*Percent of total*	*Percent*
Tobacco and wheat	Vetch	2.5	0.0535	4.18	2.50	3.08	18.37	33.79	52.16	35.34	87.50	97.26
Do	Clover	2.0	0.0652	5.28	1.66	2.83	20.17	31.53	51.70	34.19	85.89	95.66
Do	None	} 0	0.0465	1.00	1.69	2.84	17.79	33.60	51.39	37.34	88.73	94.26
Corn and wheat	—do—	}	0.0446	0.63	1.66	3.14	16.49	34.46	50.95	40.13	91.08	96.51
Tobacco and wheat	Cowpeas	1.5	0.0561	1.72	1.82	3.04	18.53	34.98	53.51	36.98	90.49	97.07
Do	Soybeans	1.5	0.0525	2.03	2.42	3.28	18.62	36.87	55.49	36.29	91.78	99.51
Do	Grass	4.0	0.0477	0.59	1.90	3.20	19.30	36.31	55.61	37.01	92.62	98.31
Tobacco and oats	Vetch	2.0	0.0609	4.33	2.79	3.34	18.43	33.73	52.16	35.24	87.40	97.86
Do	Clover	1.5	0.0613	6.05	1.12	4.28	17.93	36.47	54.40	35.86	90.26	101.71
Do	None	} 0	0.0437	0.97	1.54	2.86	19.00	35.08	54.08	38.59	92.67	98.04
Corn and oats	—do—	}	0.0395	0.66	1.68	2.78	20.31	34.67	54.98	36.78	91.76	96.88
Tobacco and oats	Cowpeas	1.0	0.0518	1.42	1.38	2.27	17.43	39.39	56.82	39.88	96.70	101.77
Do	Soybeans	1.0	0.0466	1.85	1.69	2.87	21.31	38.99	60.30	34.76	95.06	101.47
Do	Grass	3.5	0.0903	0.66	2.27	3.49	18.26	38.14	56.40	40.48	96.88	103.30
Tobacco and rye	Vetch	2.5	0.0480	5.27	1.38	3.21	19.92	35.69	55.61	33.63	89.24	99.10
Do	Clover	1.5	0.0481	4.85	0.42	2.94	20.15	35.88	56.03	35.77	91.80	100.01
Do	None	} 0	0.0334	0.94	1.02	4.87	16.44	36.72	53.16	40.57	93.73	100.56
Corn and rye	—do—	}	0.0319	0.63	1.08	4.66	18.84	35.33	54.17	41.12	95.29	101.66
Tobacco and rye	Cowpeas	1.5	0.0432	1.36	0.78	3.44	17.24	36.78	54.02	39.80	93.82	99.40
Do	Soybeans	1.0	0.0434	1.59	0.96	3.25	18.69	35.43	54.12	39.09	93.21	99.01
Do	Grass	4.5	0.0410	0.74	2.01	3.14	17.95	37.25	55.20	37.36	92.56	98.48
Correlation (r) with root rot index [2]			−0.386	−0.273	−0.039	−0.009	+0.152	−0.187	−0.092	+0.166	+0.039	
Standard error			±0.090	±0.097	±0.105	±0.105	±0.103	±0.102	±0.105	±0.103	±0.105	

[1] Brown root rot absent; these plots not included in statistical analysis.
[2] These correlations calculated from original values of which the figures in this table are averages.

TABLE 3.—*Rotations, occurrence and severity of brown root rot, and carbohydrate: nitrate nitrogen ratios of tobacco rotation plots*

2-year rotation	Cover crop	Root rot index	Carbohydrate: nitrate nitrogen ratio
Tobacco and wheat	Vetch	2.5	26.3
Do	Clover	2.0	28.7
Do	None	[1] 0	
Corn and Wheat	do		
Tobacco and wheat	Cowpeas	1.5	81.8
Do	Soybeans	1.5	53.5
Do	Grass	4.0	194.1
Tobacco and oats	Vetch	2.0	32.8
Do	Clover	1.5	33.9
Do	None	[1] 0	
Corn and oats	do		
Tobacco and oats	Cowpeas	1.0	94.9
Do	Soybeans	1.0	66.0
Do	Grass	3.5	267.5
Tobacco and rye	Vetch	2.5	37.2
Do	Clover	1.5	41.1
Do	None	[1] 0	
Corn and rye	do		
Tobacco and rye	Cowpeas	1.5	94.7
Do	Soybeans	1.0	99.0
Do	Grass	4.5	221.2
Correlation (r) with root rot index [2]			+0.479
Standard error			±0.081

[1] Brown root rot absent; these plots not included in statistical analysis.
[2] This correlation calculated from original values of which the figures in this table are averages.

The coefficient of correlation between the severity of brown root rot and the total nitrogen content of the soil was found to be −0.386 with a standard error of ±0.090, which is significant (table 2). Analysis of variance indicates that the total nitrogen content varied significantly with the cover crop. Correlations between the various fractions of nitrogen, expressed as parts per million of the whole soil and the severity of brown root rot are all negative. Although some appear to be significant, they are obviously associated with variations in the total nitrogen content of the soil. The influence of this total nitrogen is overcome by a redetermination of the coefficients using the nitrogen fractions expressed as percentages of the total. These coefficients are given in table 2.

The only significant coefficient of correlation in table 2 is that between the severity of brown root rot and the nitrate nitrogen fraction, −0.273 ± 0.097. Since this coefficient is negative it is evident that low amounts of nitrate nitrogen are associated with severe brown root rot. Analysis of variance shows that variation in nitrate nitrogen with the cover crop preceding tobacco was overwhelmingly significant. However, the fallow plots on which brown root rot did not occur were quite low in nitrate nitrogen.

It will be noted in table 1 that the carbohydrate fraction of the soil was of no significance in its relation to brown root rot. If, however, values for the first sampling of each season are employed, the correlation with brown root rot is +0.311 ± 0.165. Although this correlation is not quite significant, it is interesting to note that at least in a general way a high percentage of carbohydrate in the organic matter during the early season was associated with greater severity of brown root rot.

The simple coefficient of correlation between the carbohydrate fraction and nitrate nitrogen was found to be $+0.360\pm0.092$. This correlation is based upon carbohydrate and nitrate nitrogen expressed as parts of the whole soil, and not as parts of the total organic matter and total nitrogen, respectively. The whole soil is the only common basis for those two factors.

Using this correlation coefficient, the partial correlation between the severity of brown root rot and the carbohydrate fraction, with the influence of the nitrate factor constant, was found to be -0.092, which of course is nonsignificant. However, when brown root rot is correlated with nitrate nitrogen, with the influence of the carbohydrate factor constant, the partial coefficient of correlation becomes -0.245. This is significant although a little less so than the simple correlation between brown root rot and nitrate nitrogen. This correlation might indicate that although the carbohydrate fraction might be significant in influencing the nitrate content of the soil, it had very little direct bearing upon brown root rot. The multiple correlation is more significant, being $+0.305\pm0.096$, greater than either of the simple correlations involved. This indicates that variations in the severity of brown root rot are associated with variations in these two soil constituents.

In a further study of these two constituents, the carbohydrate: nitrate nitrogen ratio for each soil was calulcated (table 3). In making these calculations it was impossible to construct ratios where analyses showed nitrate nitrogen to be absent from the soil. To expedite the work, the few soils (four in number) that contained no nitrate nitrogen were arbitrarily assigned 1 part per million of nitrate in order that ratios other than infinity could be constructed.

The correlation between the severity of brown root rot and the carbohydrate: nitrate nitrogen ratios thus constructed was found to be $+0.479\pm0.081$. This correlation is highly significant, and indicates strongly that the more severe brown root rot was associated with wide carbohydrate : nitrate nitrogen ratios.

To determine whether the carbohydrate or the nitrate in themselves influenced the correlation between brown root rot and the carbohydrate: nitrate nitrogen ratio, partial correlation coefficients were calculated. When the severity of brown root rot was correlated with the ratio, with nitrate nitrogen constant, the severity of brown root rot still varies directly with the carbohydrate: nitrate nitrogen ratio, although not so significantly as in simple correlation. When brown root rot was correlated with the ratio, with the carbohydrate fraction constant, the partial correlation coefficient was $+0.492$. With the carbohydrate content of the soil constant, the severity of brown root rot apparently would be even more highly correlated with the carbohydrate: nitrate nitrogen ratio. Thus of the two factors involved, nitrate nitrogen played by far the more important role in the severity of brown root rot, and the carbohydrate content was of secondary importance.

DISCUSSION

As was pointed out above, the negative correlation between organic matter and the severity of brown root rot seems to be only coincidental. This seems to be confirmed by the fact that although brown root rot

varied significantly with the preceding cover crop, the total organic content of the soil did not. The fallow plots, in which the organic content was very low, did not produce brown root rot. In previous work (5) brown root rot was most severe when cellulose was added, increasing the organic content of the soil. These points are definite evidence that brown root rot does not increase with decreasing organic matter in the soil, and that greater severity of brown root rot in the field is only coincidental with low organic matter.

It is noted that only two fractions of the organic matter, as determined by the system of proximate analysis employed, are correlated at all significantly with the severity of brown root rot. Those fractions are the lignin-humus and that part of the organic nitrogenous complexes calculated from nonamide hydrolized nitrogen. If highly significant, which they were not, the correlations would indicate that large percentages of nonamide complexes accompany slight severity of brown root rot, and that large percentages of lignin-humus in the organic matter accompany great severity of brown root rot. The correlations are too small to justify anything more than the barest generalizations, even though statistically they are significant.

Although a significant coefficient of correlation (-0.386 ± 0.090) is found between the total nitrogen content of the soil and the severity of the disease, and although analysis of variance shows that total nitrogen varies significantly with the previous cover crop, there are facts which minimize the importance of the total nitrogen of the soil. (1) The plots upon which no cover crops were grown (omitted from statistical analysis) and which produced no brown root rot, contained even less total nitrogen than the plots on which brown root rot was most severe. (2) The correlation of brown root rot with total organic matter probably was coincidental. Since total nitrogen is highly dependent upon and correlated with total organic matter, it follows that the variations in the percentage of total nitrogen in the soil are also probably coincidental with variations in the severity of the disease. (3) These factors are so positively correlated with each other that partial correlations with one, holding the influence of the other constant, are nonsignificant. These facts seem to indicate that neither the total organic matter content nor the total nitrogen content of the soil is highly significant in its effect upon the severity of brown root rot of tobacco.

Of the different nitrogen fractions investigated, the only association that is significant is that with nitrate nitrogen. Analysis of variance shows that the association between the nitrate nitrogen fraction and the kind of preceding cover crop is highly significant. The fact that nitrate nitrogen was low on fallow plots, which produced no brown root rot, seems to indicate, however, that large amounts of nitrate nitrogen are not necessary to prevent the disease, and that some other factor is probably involved. This other factor appears to be the carbohydrate fraction of the soil organic matter. As pointed out, the association with carbohydrate content of the organic matter is almost significant when only the first early season samplings are considered. Also, the increase in value of the multiple correlation coefficient ($+0.305 \pm 0.096$) between brown root rot and both nitrate and carbohydrate over either of the simple correlations, indicates that the carbohydrate association must have some significance. The effect

of the carbohydrate on the correlation between brown root rot and the nitrate content of the soil is shown in the partial correlations. There is a significant correlation (-0.245) between brown root rot and nitrate nitrogen when the influence of the carbohydrate fraction is held constant. Thus the carbohydrate factor does affect nitrate nitrogen, but probably does not affect brown root rot directly.

The importance of nitrate nitrogen as it might influence brown root rot is emphasized by the correlations between brown root rot and the carbohydrate: nitrate nitrogen ratio. Although the simple correlation coefficient is very significant ($+0.479\pm0.081$), partial correlations between brown root rot and the ratio, when the influence of the constituent parts, either carbohydrate or nitrate, is held constant, shows that the nitrate nitrogen rather than the carbohydrate. is the most important factor influencing the correlation between brown root rot and the carbohydrate: nitrate nitrogen ratio.

No definite minimum value for nitrate nitrogen below which brown root rot should occur, can be designated. However, it would seem that the critical nitrate value, if any, would be dependent in some manner upon the carbohydrate: nitrate nitrogen ratio of the soil. To test this supposition, the partial correlation between the severity of brown root rot and nitrate nitrogen was determined, with the influence of the carbohydrate: nitrate nitrogen ratio held constant. The value was found to be but -0.044, as compared to -0.292 for the simple correlation between brown root rot and nitrate nitrogen, as parts of the whole soil. Evidently the influence of variations in nitrate nitrogen upon the severity of brown root rot is dependent upon the influence of variations in the carbohydrate: nitrate nitrogen ratio of the soil.

The results of this work seem to confirm those obtained by Thomas (6) in his earlier work on nitrate nitrogen and nitrification in relation to growth of tobacco and to brown root rot. However, the work of Thomas apparently indicates that carbohydrate material has a more important direct bearing upon the occurrence and severity of the disease than does the present work. The statistical analysis here presented shows that it is highly important only in its ratio to nitrate nitrogen. It shows further that the ratio is of significance chiefly in its influence upon the association between brown root rot and the nitrate nitrogen content of the soil. Nitrate nitrogen is the only soil constituent in itself found to be highly significant in its direct influence upon the severity of brown root rot of tobacco.

SUMMARY AND CONCLUSIONS

A study was made of biochemical soil factors which might influence the disease known as brown root rot of tabacco. A number of soils from tobacco rotation plots at upper Marlboro, Md., were investigated. The amount of organic matter in each was determined as well as the following constituents of the organic matter: The benzene-alcohol soluble fraction, the carbohydrate material, organic nitrogenous complexes as calculated from amide, nonamide acid-hydrolyzed, hydrolysis-resistant nitrogen, and lignin-humus. The total soil nitrogen and its biochemical distribution was determined, the fractions investigated being nitrate nitrogen, water-soluble nitrogen other than nitrate, ammonia nitrogen, amide nitrogen, nonamide acid-hydrolyzed

nitrogen, and resistant or nonhydrolyzed nitrogen. The severity of brown root rot was estimated by direct examination of the tobacco roots, and a root rot index established. The laboratory results were analyzed statistically in conjunction with the root rot indices, in order to determine what constituents of the soil organic matter or what fractions of soil nitrogen might be associated with the severity of brown root rot.

Nitrate nitrogen was the only single soil constituent found to be very significant in its influence upon the severity of brown root rot. The coefficient of correlation between nitrate nitrogen, as parts per million of the soil, and the severity of the disease was found to be -0.292 ± 0.096. When nitrate nitrogen is expressed as percent of the total nitrogen, this coefficient is -0.273 ± 0.097. The carbohydrate fraction alone was of no significance, but the correlation between brown root rot and the carbohydrate: nitrate nitrogen ratio is the most significant encountered, being $+0.479 \pm 0.081$. Partial correlations indicate that the influence of variations in nitrate nitrogen upon the severity of brown root rot was very largely dependent upon the influence of variations in the carbohydrate: nitrate nitrogen ratio.

It is concluded that the severity of brown root rot is associated negatively with the nitrate nitrogen content and with the carbohydrate-nitrate nitrogen ratio of the soil. The preceding crop affects the succeeding tobacco crop indirectly by its influence upon the carbohydrate: nitrate nitrogen ratio, and more directly by its effect upon the nitrate nitrogen content of the soil.

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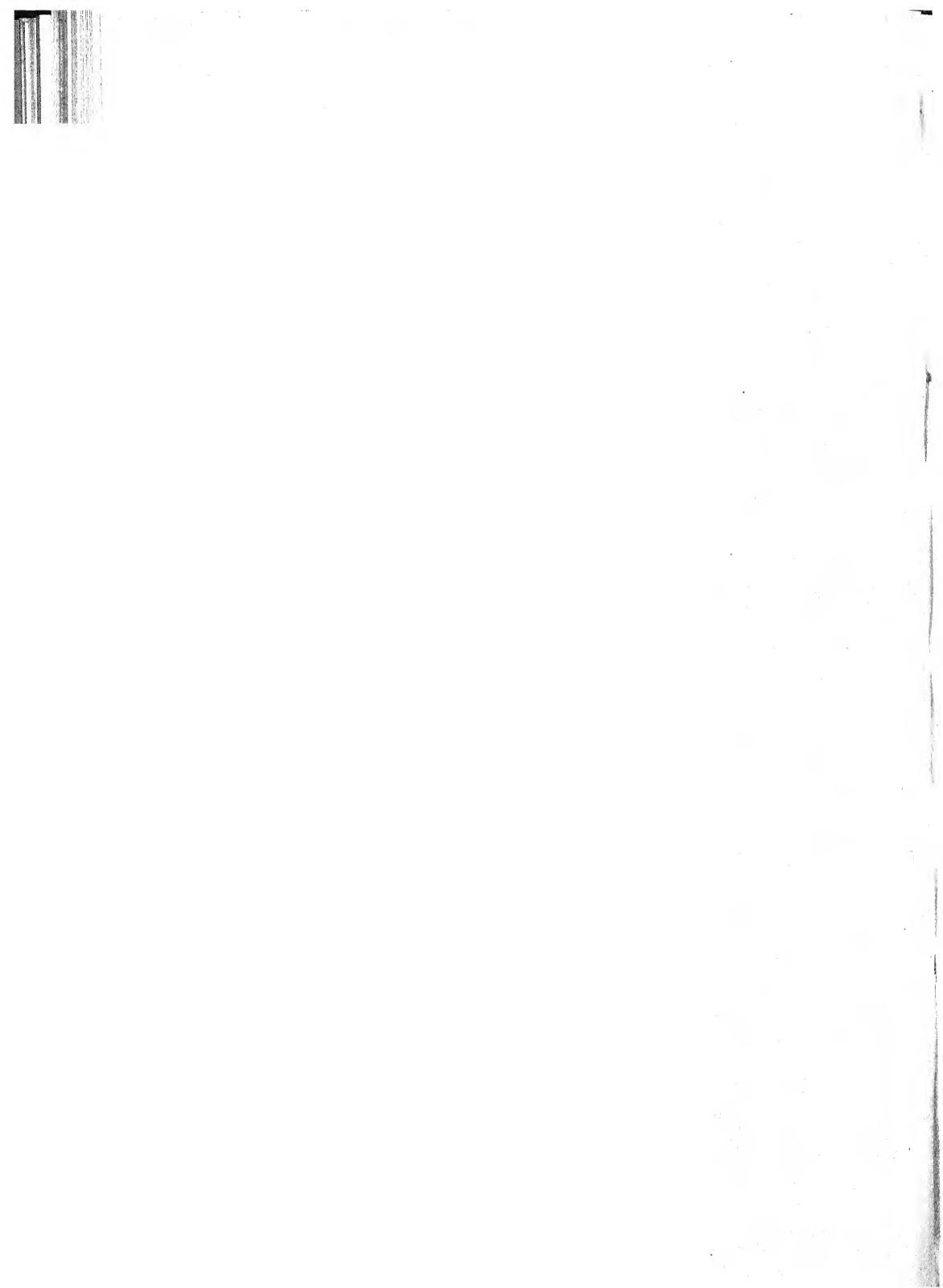
RESPIRATION OF CITRUS FRUITS AFTER HARVEST [1]

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INTRODUCTION

It is generally assumed that the rate at which a fruit respires after harvest is a measure of the rate at which it is deteriorating. If this assumption is correct, high respiratory activity should be associated with rapid deterioration or short storage life. Respiration is a chemical process, and, as with other chemical reactions, the rate increases greatly with temperature. On the other hand, the storage life of fruit does not necessarily decrease with increased temperature, as certain physiological disorders such as pitting of oranges, lemons, and grapefruit and membranous stain of lemons are associated with low temperatures, so that a longer commercial life of these fruits at the higher temperatures is associated with a more rapid rate of respiration. However, respiratory activity does indicate the rate at which the elaborated foods are being oxidized and the fruit is deteriorating in food value. This loss of food value may be extremely slow at low temperatures, but it assumes appreciable proportions at high temperatures.

The respiratory rate is also an indirect measure of the heat evolved by the oxidation of the carbon present, principally as sugar and acid in citrus fruits. This heat evolved is of particular interest from the standpoint of refrigeration of the fruit during transportation and storage.

A preliminary report on the relation of temperature to the respiration of certain fruits has been made (6); [2] this paper presents further data on the relation of temperature and other factors to the respiration of certain citrus fruits.

MATERIAL AND METHODS

The respiration determinations were made at the experimental cold-storage laboratory of the Bureau of Plant Industry, Soils, and Agricultural Engineering, near Washington, D. C., on oranges (Citrus sinensis (L.) Osbeck) and grapefruit (C. paradisi Macfad.) from Florida and California and on lemons (C. limonia Osbeck) from California. In the earlier experiments the Florida fruit was obtained on the Washington, D. C., market. In the later experiments the fruit was shipped to the experimental laboratory by unrefrigerated express directly from the point of production.

The respiration determinations were made by a method previously described (7), the apparatus being set up in the different constant-

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temperature rooms.　By this method the amount of oxygen consumed, as well as the amount of carbon dioxide evolved, was measured. The determinations were usually run in duplicate at each temperature, and each run extended over a period of approximately 24 hours, except that at the low temperatures (32° and 40° F.) each run was generally of approximately 48 hours.　(On the graphs such 2-day runs at the low temperatures are plotted as 1-day runs.)

At each temperature a period of 12 to 24 hours was allowed for the fruit to come to the temperature of the room before the determinations were started.

Results were tested for statistical significance by analysis of variance.

EFFECT OF TEMPERATURE AND VARIOUS OTHER FACTORS ON RESPIRATION

FRUIT TESTED

FLORIDA ORANGES

When the earlier tests (1929–30) were made on Florida oranges, facilities were not available to determine the respiration rate at all temperatures simultaneously; consequently, determinations were made on each lot at only a few of the temperatures.　However, on each lot except No. 5 the rate was determined at 60° F., as a standard for comparison.　The rates of evolution of carbon dioxide by the various lots at 60° did not differ significantly (table 1) except for one lot that was severely russeted; therefore, it seemed permissible to compare the averages for all temperatures, even though comparable fruit was not used throughout at all temperatures.　The results are plotted in figure 1, *A*, for the carbon dioxide evolved, and in figure 2, *A*, for the respiratory ratio of the volume of carbon dioxide evolved to the volume of oxygen consumed.　Generally runs were made at each temperature for at least 4 days, and the carbon dioxide determinations are plotted to show the change in rate with time.　As will be seen (fig. 1, *A*), there was no appreciable change in rate with time at the lower temperatures (32°–50°).　At 60° there was a slight increase and at the higher temperatures a decrease.　The decrease was very marked at 90° and 100°.

Although four determinations were made on Washington Navel oranges at each of the temperatures, duplicate determinations at 32°F. were averaged; hence only two determinations are shown (fig. 1, *C*) for this temperature.

The curves show an increase in rate of respiration with each rise in temperature.　At temperatures above 70° F. the carbon dioxide evolved generally increased to a greater extent than the oxygen consumed, with a consequent increase in the respiratory ratios (fig. 2, *A*). It is generally assumed that a ratio of 1.0 represents the complete oxidation of a hexose sugar to carbon dioxide and water and that a ratio of 1.33 represents the complete oxidation of citric acid.　Intermediate ratios might represent the respiration of various proportions of sugar and acid (see table 10), whereas ratios above 1.33 probably are due to partial intramolecular respiration.　Further consideration of the significance of the respiratory ratios will be found under Discussion (p. 353).

TABLE 1.—*Effect of temperature and other factors on respiratory activity of Florida oranges, 1929–30 and 1941–42*

[Respiratory activity expressed as miligrams of CO_2 per kilogram-hour]

Lot No.[1]	Date	Respiratory activity (CO_2 evolved)[2] at indicated temperature (° F.)								
		32	40	50	60	70	80	90	100	110
	1929	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
	Dec. 3–7		5.9		20.4					
			6.5		21.0					
1	Dec. 12–14	3.0			21.9		35.7			
		3.0			19.5		35.0			
	Dec. 17–23	3.0			21.7					
		3.1			20.5					90.6
	1930									
2	Jan. 13–20	3.2			16.6				70.3	106.1
		3.2			17.7				75.7	106.5
	Jan. 20–25				21.9			44.8		
					21.9			45.2		
3	Jan. 27–Feb. 1			11.9	19.9	29.0		58.2		
				12.6	22.9	29.7		42.1		
	Feb. 3–8				23.5	31.7		44.8		
						31.2		44.0		
4	Feb. 17–24		6.1		23.5					
			7.1		19.9					
5	Mar. 10–15					28.5				
						29.3				
6	Apr. 7–18			18.6					66.8	
				20.2					63.7	
Average, 1929–30		3.1	6.4	12.3	20.7	29.9	35.4	46.5	69.1	101.1
	1941									
7a	Nov. 28–Dec. 3	2.8		17.9		41.0				
7b	do	3.0		18.5		38.0				
8a	Dec. 6–12	2.1		15.7		30.8				
		2.2		14.9		30.4				
8b	do	2.0		13.0		26.6				
		2.0		12.5		27.1				
	1942									
9a	Mar. 24–Apr. 1	4.2		19.9		42.8				
		4.7		21.9		40.1				
9b	do	3.5		20.5		37.0				
		3.5		18.3		37.2				
10a	Apr. 28–May 6	2.9		17.8		37.3				
		3.2		17.2		41.6				
10b	do	2.6		15.0		33.2				
		2.7		15.3		35.0				
11a	June 2–8	2.5		12.7		27.4				
		2.4		12.1		26.8				
11b	do	2.2		10.9		24.5				
		1.9		11.5		23.8				
Average, 1941–42		2.8		16.1		34.0				

[1] Lot 1, oranges purchased on Washington, D. C., market, Dec. 3, 1929; variety and previous history unknown. Lot 2, oranges purchased on Washington, D. C., market, Jan. 12, 1930; variety unknown; fruit heavily russeted. Lot 3, oranges shipped directly from Florida; received Jan. 18, 1930; variety unknown. Lot 4, oranges shipped directly from Nocatee, Fla.; received Feb. 15, 1930; variety unknown. Lot 5, Valencia oranges shipped Feb. 20, 1930, directly from Bradenton, Fla. Lot 6, oranges shipped Mar. 18, 1930, directly from Frostproof, Fla.; variety unknown. Lot 7, Washington Navel oranges on sour orange (*Citrus aurantium* L.) rootstock in heavy hammock soil, shipped directly from Citra, Fla., Nov. 24, 1941; received Nov. 27. Lot 7a, fruit from low ground; solids, 10.6 percent; acid, 0.62 percent; ratio, 17.1. Lot 7b, fruit from hilltop; solids, 10.7 percent; acid, 0.78 percent; ratio, 13.7. Lot 8, Parson Brown oranges grown on Norfolk fine sand, shipped directly from Orlando, Fla., Dec. 2, 1941; received Dec. 5. Lot 8a, sour orange rootstock; solids, 10.85 percent; acid, 1.42 percent; ratio, 7.6. Lot 8b, rough lemon (*C. limonia*) rootstock; solids, 9.9 percent; acid, 0.94 percent; ratio, 10.5. Lot 9, Valencia oranges (early picking) grown in Norfolk fine sand, shipped from Windermere, Fla., Mar. 9, 1942; received Mar. 12; held at 32° F. until beginning of experiment. Lot 9a; sour orange rootstock; solids, 13.2 percent; acid, 1.81 percent; ratio, 7.3. Lot 9b, rough lemon rootstock; solids, 11.3 percent; acid, 1.53 percent; ratio, 7.4. Lot 10a, same as lot 9a but picked and shipped Apr. 20, 1942; solids, 13.2 percent; acid, 1.42 percent; ratio, 9.3. Lot 10b, same as lot 9b but picked and shipped Apr. 20, 1942; solids, 12.1 percent; acid, 1.16 percent; ratio. 10.4. Lot 11a, same as lot 9a but picked and shipped May 29, 1942; solids, 13.4 percent; acid, 1.08 percent; ratio, 12.4. Lot 11b, same as lot 9b but picked and shipped May 29, 1942; solids, 11.0 percent; acid, 0.93 percent; ratio, 11.8.

[2] The 2 figures given for each date period represent determinations on duplicate samples of fruit.

The respiratory ratios for these Florida oranges (fig. 2, *A*) averaged about 1.0 at 32° F. and increased to about 1.20 at 50° to 70°. There was a further increase above 70° to a maximum of 2.0 at 100°. This would indicate that sugar was respired at 32° but that a higher per-
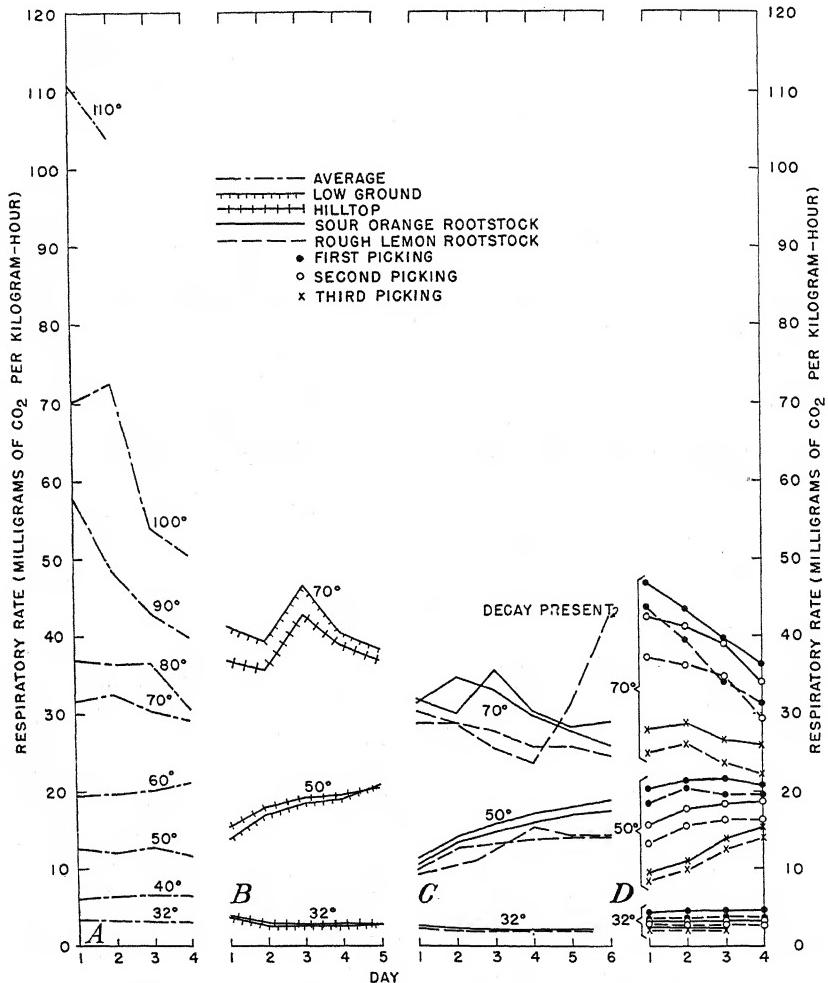
FIGURE 1.—Effect of temperature (°F.) on respiratory activity of oranges grown in Florida: *A*, Average for various lots, 1929–30 season; *B*, Washington Navel oranges from low-ground and hilltop soils, 1941 season; *C*, Parson Brown oranges from trees grown on sour orange and rough lemon rootstocks, 1941 season; *D*, three pickings of Valencia oranges from trees grown on sour orange and rough lemon rootstocks, 1942 season.

centage of acid than of sugar was respired at 50° to 70° or that possibly the sugar was incompletely oxidized, with the formation of intermediate products such as ethylene or alcohol. At 90° and above, the ratios indicated that considerable intramolecular respiration was taking place.

In the tests made during the season of 1941–42, only temperatures of 32°, 50°, and 70° F. were used and the influence of rootstock and maturity was investigated.   There was a greater variation among
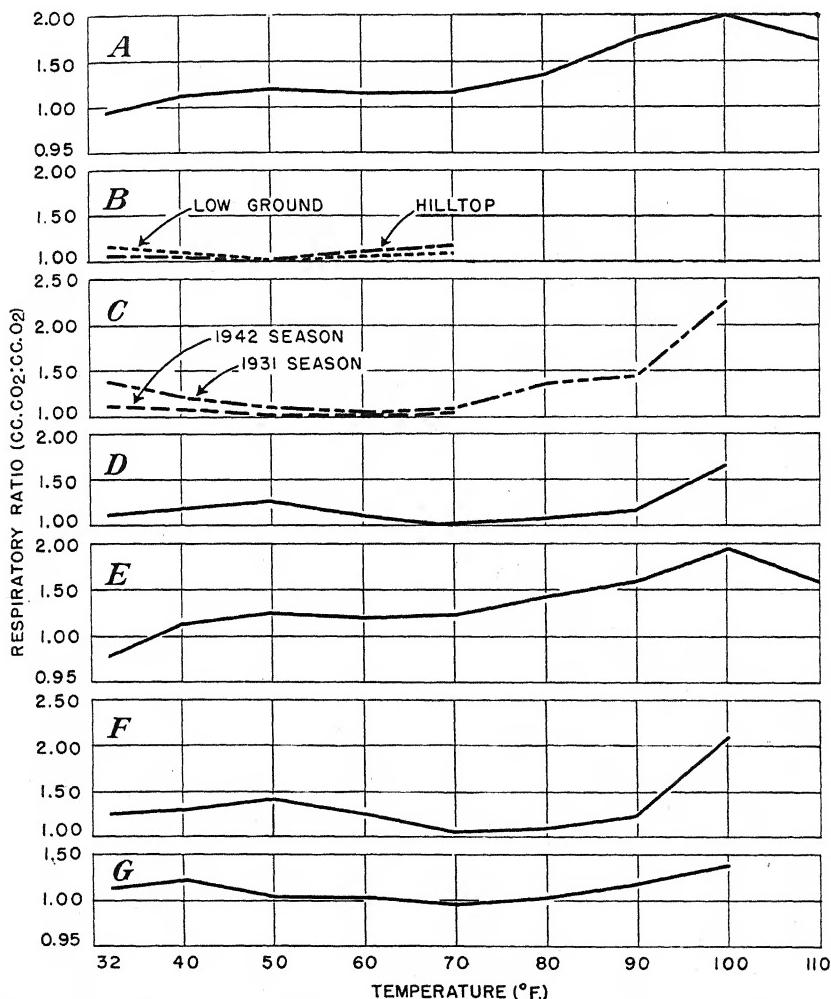
FIGURE 2.—Effect of temperature on respiratory ratio (cc. CO_2: cc. O_2) of Florida and California oranges, grapefruit, and lemons: *A*, Average for various lots of oranges grown in Florida, 1929–30 season; *B*, Washington Navel oranges grown in Florida, 1941 season; *C*, Washington Navel oranges grown in California; *D*, Valencia oranges grown in California, 1932 season; *E*, average for various lots of grapefruit grown in Florida, 1929–30 season; *F*, Marsh grapefruit grown in California; *G*, Eureka lemons grown in California.

these lots at a given temperature (table 1) than in the lots previously tested.   Thus, at 32° the carbon dioxide evolved ranged from less than 2.0 to more than 4.0 mg. per kilogram-hour.   The average of all lots at 32° for the season of 1941–42 was 2.8 mg.   This was somewhat less than for the earlier season (1929–30).   On the other hand,

the respiratory rate increased more with higher temperatures in 1941–42 than in 1929–30. The Washington Navel oranges (table 1, lot 7, and fig. 1, *B*) had fairly high respiratory activity at 50° and 70° with no consistent difference due to soil type.

Parson Brown oranges (table 1, lot 8, and fig. 1, *C*) were intermediate to low in respiratory activity. The rate averaged about 15 percent higher in fruit grown on sour orange rootstocks than in fruit grown on rough lemon rootstocks. The higher respiratory rate with the sour orange rootstock was associated with a higher concentration of both sugar and acid in the juice. The respiratory activity of Valencia oranges (table 1, lots 9–11, and fig. 1, *D*) varied greatly and was influenced by both rootstock and maturity. As in Parson Brown, the respiratory rate was consistently higher in Valencia oranges grown on sour orange rootstock than in those grown on rough lemon rootstock, and the sugar and acid concentrations of the juice were considerably higher in oranges grown on the sour orange rootstock. The respiratory rate was highest in the fruit picked at the beginning of the commercial harvest season (table 1, lot 9) and decreased in the later pickings. The average rate for the midseason picking (lot 10) was about 88 percent as high as that for the early picking (lot 9), and the average rate for the late picking (lot 11) was only about 62.5 percent as high as that for the early picking (lot 9). There was no reduction in the sugar concentration in the later pickings to account for the lower respiratory rates, although there was a reduction in the acid concentration.

CALIFORNIA ORANGES

The respiration rates of Washington Navel and Valencia oranges are presented in figure 3, *A* and *C*, for the 1931 and 1932 seasons, respectively. The Washington Navel oranges, shipped from San Dimas, Calif., on March 6, 1931, by unrefrigerated express, arrived at the cold-storage laboratory on March 11 and were immediately weighed into samples and placed at the various temperatures. Respiration determinations started on March 12 after the fruit had come to the desired temperatures. The runs at 32° F. were for 2-day periods, and those at the higher temperatures for 1-day periods. The Valencia oranges, also from San Dimas, were shipped by unrefrigerated express on June 29, 1932, arrived at the cold-storage laboratory on July 5, and were placed at 32° F. until used. Respiration determinations at temperatures of 32° to 60° were started on July 8; those at 70°, 90°, and 100°, on July 18; and those at 80°, on July 25. The runs at 32° and 40° were for periods of 2 days but are plotted as 1-day periods on the chart. At all other temperatures the runs were for 1-day periods.

As in Florida oranges, there was a decrease with time in the respiratory rates of the California Washington Navel and Valencia oranges at 70° to 100° F. (fig. 3, *A* and *C*). In the Washington Navel oranges there was also a slight decrease at 60° but some increase at 50° and less at 40°. In the Valencia oranges the rate remained fairly constant at 60° and 50°, with a slight increase at 40°. In both varieties the rate remained rather constant at 32°.

For the more recent experiments, the Washington Navel oranges were picked and shipped from Corona, Calif., on March 6, 1942, and arrived at the cold-storage laboratory on March 12. The oranges were kept at 32° F. until the determinations were started on March 17.

The fruit came from two different groves, and determinations were made on duplicate lots from each grove. The juice of the oranges from one grove averaged 13.0 percent of soluble solids and 1.22 percent of citric acid; that from the other grove averaged 11.9 and 1.03 percent of soluble solids and citric acid, respectively. Although the fruit from the two groves differed in the composition of the juice, it did not differ significantly in respiratory activity; the points on the curves (fig. 3, *B*) represent the averages for all four lots at each temperature.
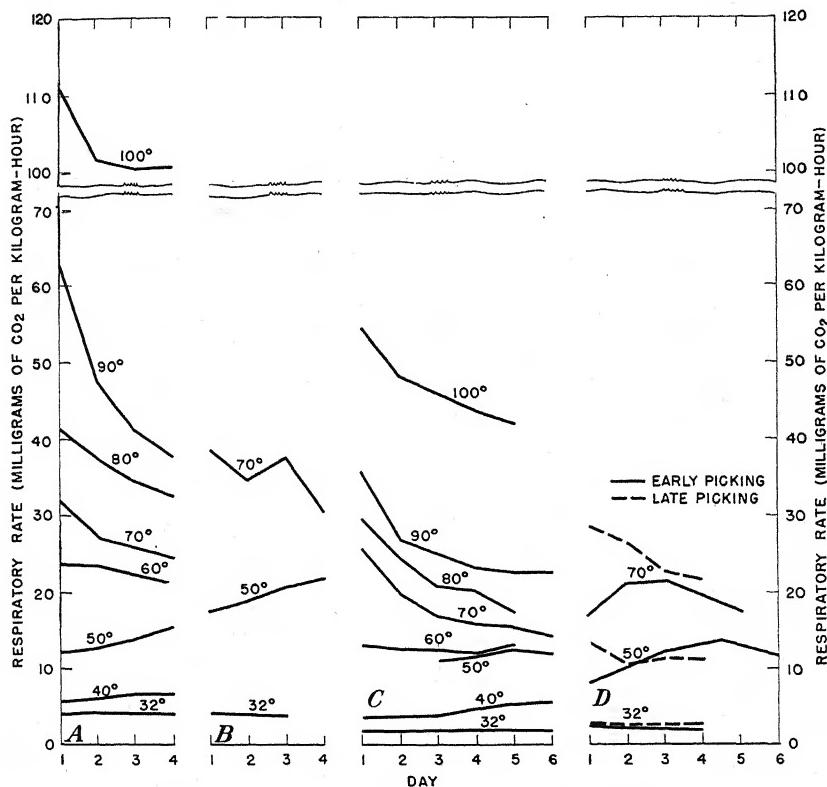
FIGURE 3.—Effect of temperature (° F.) on respiratory activity of oranges grown in California: *A*, Washington Navel oranges, 1931 season; *B*, Washington Navel oranges, 1942 season; *C*, Valencia oranges, 1932 season; *D*, early and late pickings of Valencia oranges, 1942 season.

As in the earlier tests, the rate of respiration remained constant at 32° F., increased with time at 50° and decreased at 70°. The average rate at 32° was 3.9 mg. of carbon dioxide per kilogram-hour, as in the 1931 season. However, at 50° and 70° the rate was considerably higher in 1942 than in 1931 (fig. 3, *A* and *B*).

Two pickings of Valencia oranges were made in a grove near Corona, Calif. The first picking, representing early midseason fruit, was made on July 7, 1942, was shipped by unrefrigerated express, and was received on July 14. The second picking, representing late maturity, was made from the same grove on October 1, 1942, and was received on October 7. The juice in oranges of the

first picking had 10.1 percent of soluble solids, 0.66 percent of citric acid, and a solids-acid ratio of 15.3. The late picking had 11.5 percent of soluble solids, 0.68 percent of acids, and a solids-acid ratio of 16.9. The late picking was somewhat higher in soluble solids than the first picking but did not differ appreciably from the first picking in respiratory activity except at 70° F., where it was considerably higher. At 32° and 50° the rates were approximately the same as the earlier (1932) results (fig. 3, *C* and *D*).

The average rate of respiration of the Washington Navel oranges from Florida (fig. 1, *B*) was about the same as that found in 1942 for California Navel oranges (fig. 3, *B*) at 32°, 50°, and 70° F. However, the averages for the Valencia oranges from California (fig. 3, *C* and *D*) were consistently lower than for the Florida Valencia oranges, except the late picking of Florida Valencia oranges, which was also low (fig. 1, *D*).

There was no significant difference in respiratory activity between Washington Navel oranges from Florida and early or midseason Valencia oranges from Florida. However, the Valencia oranges from California respired at a lower rate than the Washington Navel oranges from California. Gunn and Nedvidek[3] found the respiratory rates of California Valencia oranges at 70° and 100° F. to be even lower than those reported here. At 31° to 42° Trout et al. (*19*) recorded respiratory rates of Valencia and Washington Navel oranges in Australia about the same as those reported here.

In all the lots of oranges the carbon dioxide evolution increased to a greater extent at the higher temperatures (90° F. and above) than the oxygen intake, with a consequent increase in the respiratory ratio (fig. 2, *A*, *C*, and *D*). In all lots considerable intramolecular respiration was indicated by the high respiratory ratios at 100° and to a less extent by those at 90°. The ratios at the lower temperatures were generally between 1.33 and 1.00, but there was no consistent relation of the ratios to temperature below 90° in the various lots.

FLORIDA GRAPEFRUIT

As with Florida oranges, different lots of Florida grapefruit were used during the 1929–30 season at the different temperatures, but samples of nearly all lots at 60° F. were used as standards. The data for the various lots are presented in table 2. There was a greater difference between duplicate samples of grapefruit than of oranges, doubtless because of the small number of fruit used per sample. Average respiratory rates for each temperature are shown in figure 4, *A*. As with oranges, the rate decreased with time at the higher temperatures, and the steepness of the curves generally increased with increased temperature. At 50° and below, there was relatively little change in rate with time.

The respiratory rate of these lots of grapefruit (table 2) at 32° F. averaged about 2.6 mg. of carbon dioxide per kilogram-hour and increased to nearly 70 mg. at 110°. As with oranges, the rate of carbon dioxide evolution increased with temperature to a greater extent than the oxygen intake, with a consequent increase in the ratio of carbon dioxide to oxygen (fig. 2, *E*). The respiratory ratio indicated some

[3] GUNN, A., and NEDVIDEK, R. D. COLOR INVESTIGATION OF CITRUS FRUITS. STUDIES ON RESPIRATION OF VALENCIA AND NAVEL ORANGES. Calif. Fruit Growers Exch. Res. Dept. File 25, July to Dec. 1929. [1930.] [Processed.]

intramolecular respiration at 80°, increasing to a maximum at 100°, with a respiratory ratio of nearly 2.0.

TABLE 2.—*Effect of temperature and other factors on respiratory activity of Florida grapefruit, 1929–30 and 1942*

[Respiratory activity expressed as milligrams of CO_2 per kilogram-hour]

Lot No.[1]	Date	Respiratory activity (CO_2 evolved)[2] at indicated temperature (°F.)								
		32	40	50	60	70	80	90	100	110
		Milligrams	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
	1929									
	Dec. 3–7 {	......	3.3	......	10.0	......	......	......	......	......
		......	......	......	11.6	......	......	......	......	......
1	Dec. 9–14 {	2.0	......	......	10.0	......	21.1	......	......	......
		2.2	......	......	11.0	......	16.9	......	......	......
	Dec. 17–23 {	2.2	......	......	10.7	......	......	......	......	......
		......	......	......	10.1	......	......	......	......	54.6
	1930									
2	Jan. 6–13 {	2.2	......	......	11.4	......	......	......	51.4	81.6
		2.1	......	......	12.7	......	......	......	51.4	81.6
	Jan. 20–25 {	......	......	6.5	14.9	......	......	23.0	......	......
		......	......	7.3	10.5	......	......	27.5	......	......
3	Jan. 27–Feb. 2 {	......	......	......	11.9	15.2	......	31.1	......	......
		......	......	......	14.0	14.0	......	25.4	......	......
	Feb. 3–8 {	......	......	......	16.8	18.0	......	28.3	......	......
		......	......	......	13.6	......	......	29.6	......	......
4	Feb. 10–17 {	......	5.4	......	15.3	......	......	......	......	......
		......	5.9	......	16.1	......	......	......	......	......
	Feb. 22–Mar. 3 {	......	......	......	......	19.5	......	......	......	......
		......	......	......	......	17.5	......	......	......	......
5	Mar. 3–8 {	......	......	......	......	14.7	......	......	......	......
		......	......	......	......	12.7	......	......	......	......
	Apr. 19–25 {	3.4	......	......	18.1	......	......	......	......	......
		4.3	......	......	......	......	......	......	......	......
Average, 1929–30		2.6	4.5	6.9	13.2	16.0	19.0	27.5	51.4	68.1
	1942									
6a	Jan. 16–23 {	2.0	......	8.4	......	14.7	......	......	......	......
		2.0	......	7.6	......	12.0	......	......	......	......
6b	Jan. 27–Feb. 2 {	1.8	......	7.5	......	21.8	......	......	......	......
		1.9	......	8.1	......	16.9	......	......	......	......
7a	Jan. 16–23 {	1.8	......	8.1	......	14.3	......	......	......	......
		1.8	......	8.8	......	16.3	......	......	......	......
7b	Jan. 27–Feb. 2 {	1.7	......	7.2	......	18.0	......	......	......	......
		1.8	......	6.1	......	17.6	......	......	......	......
8	Feb. 17–23 {	1.9	......	8.7	......	24.1	......	......	......	......
		1.8	......	9.0	......	25.2	......	......	......	......
9	do {	2.4	......	9.3	......	26.0	......	......	......	......
		2.4	......	9.7	......	25.1	......	......	......	......
Average, Jan.–Feb. 1942		1.9	......	8.2	......	19.3	......	......	......	......

[1] Lot 1, grapefruit purchased on Washington, D. C., market, Dec. 3, 1929; variety and previous history unknown. Lot 2, grapefruit purchased on Washington, D. C., market, Jan. 2, 1930; shipped from Manavista, Fla.; variety unknown. Lot 3, grapefruit shipped directly from Florida; variety unknown. Lot 4, grapefruit bought on Washington, D. C., market, Jan. 10, 1930; fruit (Brogdexed) from Orlando, Fla. Lot 5, grapefruit shipped directly from Arcadia, Fla., Feb. 14, 1930. Lot 6, Marsh grapefruit grown in Portsmouth series (low hammock) soil, shipped directly from Vero Beach, Fla., Jan. 12, 1942. Lot 6a, on sour orange rootstock; solids, 10.0 percent; acid, 1.26 percent; ratio, 7.9. Lot 6b, on rough lemon rootstock grown in Norfolk fine sand; solids, 8.4 percent; acid, 1.26 percent; ratio, 6.7. Lot 7a, same as lot 6a but Duncan variety; solids, 10.2 percent; acid, 1.58 percent; ratio, 6.5. Lot 7b, same as lot 6b but Duncan variety; solids, 9.2 percent; acid, 1.33 percent; ratio, 6.9. Lot 8, Foster (pink) grapefruit on sour orange rootstock grown in Parkwood series soil, shipped from Bradenton, Fla., Feb. 11, 1942; solids, 10.3 percent; acid, 1.07 percent; ratio, 9.6. Lot 9, Thompson (pink) grapefruit, same as lot 8; solids, 9.0 percent; acid, 1.21 percent; ratio, 7.4.

[2] The 2 figures given for each date period represent determinations on duplicate samples of fruit.

Marsh (seedless) and Duncan (seeded) grapefruit were obtained from two sources in Florida. The first shipment of both varieties picked on January 12, 1942, was obtained from growers on the eastern coast of Florida near Vero Beach. Grapefruit from this section is

tender, thin-skinned, and of high quality. It was grown on sour orange rootstock in a low hammock soil which has a high organic-matter content. Another lot of the same varieties picked a week later was obtained from near Davenport, Fla. This was grown on rough lemon rootstock in a Norfolk fine sand which has a low organic-matter content. The results for these lots, together with those for two varieties of pink grapefruit (Foster and Thompson), are shown in table 2 and figure 4, *B* and *C*.

There was no consistent difference in respiratory activity between the two varieties Marsh and Duncan or because of the sources and
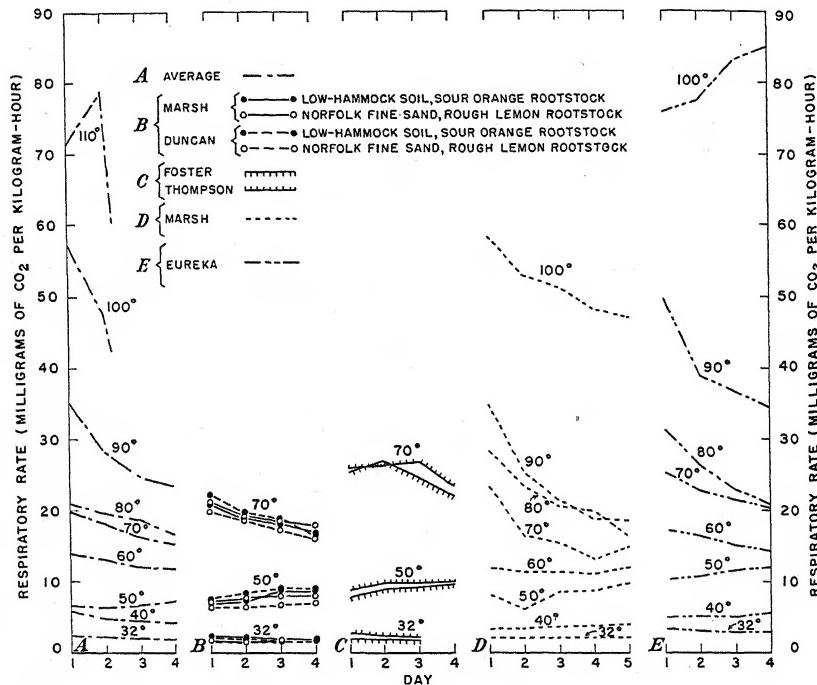
FIGURE 4.—Effect of temperature (°F.) on respiratory activity of grapefruit and lemons: *A*, Average for various lots of grapefruit grown in Florida, 1929–30 season; *B*, grapefruit grown in Florida, 1942 season; *C*, average for various lots of grapefruit from trees grown at Bradenton, Fla., on sour orange rootstocks, 1942 season; *D*, Marsh grapefruit grown in California, 1932 season; *E*, Eureka lemons grown in California.

rootstocks from which they were obtained, although in the fruit from Vero Beach the solids content was somewhat higher on the sour orange rootstock. The pink varieties (Foster and Thompson) respired at a slightly higher rate at 32° and 50° F. and at a considerably higher rate at 70° than did fruit of the Marsh and Duncan varieties. The higher rate for the pink varieties was not associated with a higher content of soluble solids or acidity in the juice of the fruit.

CALIFORNIA GRAPEFRUIT

The effect of temperature on the respiration of Marsh (seedless) grapefruit from California is shown in figure 4, *D*. This lot of grape-

fruit was shipped by unrefrigerated express from San Dimas on June 29, 1932, and arrived at the cold-storage laboratory on July 5. The fruit was stored at 32° F. until used. Samples were weighed and placed at temperatures of 32° to 60° on July 9, and respiration determinations were started on July 11. Other samples were weighed and placed at 70° and 90° on July 18, and determinations were started July 19. At 80° and 100° the corresponding dates were July 25 and 26. At the higher temperatures, the respiration rates (fig. 4, *D*) were similar to those for Florida grapefruit (fig. 4, *A*). With California grapefruit there was relatively little difference in respiratory activity between 80° and 90°, and with Florida grapefruit there was little difference between 70° and 80°. The respiratory ratio of the California grapefruit at 100° (fig. 2, *F*) was high (2.1), indicating considerable intramolecular respiration. The ratios at the lower temperatures did not correspond with those shown (fig. 2, *E*) for Florida grapefruit.

CALIFORNIA LEMONS

Five lots of Eureka lemons representing several stages of maturity were obtained from California in three different years (table 3). The light-green lemons of 1931 were shipped from San Dimas on April 10 and were received at the cold-storage laboratory 5 days later; the respiration determinations at 32° to 80° F. were begun the following day. At 90° and 100° the determinations were started on May 23 and 29, respectively, and the lemons were held at 32° until the experiments began. The silver lemons of 1931 were shipped from California on May 8 and received 5 days later. They were held at 32° until tested. Samples were weighed and placed at temperatures of 32° to 80° on May 15, and the determinations were started on May 18. At 90° and 100° the determinations were made at the same time as those with the light-green lemons. The tree-ripe lemons of 1932 were shipped from San Dimas about April 18, received April 23, and held at 40° until tested. At 32° to 70° the respiration determinations were started April 25, at 80° on April 27, and at 90° and 100° on May 5. Both the silver and the green lemons of 1933 were shipped from San Dimas on April 24. The fruit was received about 5 days later and held at 40° until tested. Respiration determinations on the silver lemons were started at all temperatures (32° to 90°) on May 15. With the green lemons, the determinations were started at 32° on May 15 and at the higher temperatures (40° to 90°) on May 22.

Although the respiration of lemons at several stages of maturity is shown in table 3, the only comparable lots are those of 1933, in which fruits of the two stages of maturity were obtained at the same time and from the same source. In these two lots the green lemons were found to respire in most cases more rapidly than the silver lemons at the various temperatures, on the basis of both carbon dioxide and oxygen determinations. The differences were statistically significant. The shipment of light-green lemons received in 1931 contained some that were dark green. The respiratory rates for these were determined at 60°, 80°, 90°, and 100° F. These data being incomplete with respect to temperatures used, are not presented in table 3. At the temperatures employed, the dark-green lemons respired consistently faster than the light-green lemons (average 9.1 percent higher). The results indicate that in lemons the

respiratory rate decreases with increased maturity. A similar relation has been shown for Valencia oranges picked at different times (table 1).

TABLE 3.—*Effect of temperature on respiratory activity of California Eureka lemons of various stages of maturity, 1931–33*

[Respiratory activity expressed as milligrams of CO_2 per kilogram-hour; O_2 measured as cubic centimeters per kilogram-hour]

Year	Maturity of fruit	Temperature	Respiratory activity (CO_2 evolved) during run [1] No. — 1	2	3	4	Average (1–4)	O_2 consumed, average (1–4)	Ratio of volume of CO_2 to volume of O_2 $\left(\dfrac{cc.\ CO_2}{cc.\ O_2}\right)$
		° F.	Milligrams	Milligrams	Milligrams	Milligrams	Milligrams	Cubic centimeters	
1931	Silver	32	2.8	2.7	2.6	2.6	2.7	1.1	1.25
	Light green	32	2.8	2.3	2.2	2.2	2.4	1.0	1.22
1932	Tree-ripe	32	2.5	2.2	2.0	2.3	2.2		
1933	Green	32	4.4	4.2	4.2	3.8	4.1	2.0	1.05
	Silver	32	4.5	4.0	3.6	3.7	3.9	1.9	1.05
Average		32	3.4	3.1	2.9	2.9	3.1	1.5	1.14
1931	Silver	40	3.8	3.8	3.2	3.9	3.7	1.6	1.18
	Light green	40	2.5	3.0	2.8	2.9	2.8	1.2	1.19
1932	Tree-ripe	40	3.0	3.0	3.1	3.7	3.2	1.4	1.17
1933	Green	40	9.0	8.5	8.2	8.6	8.6	3.2	1.37
	Silver	40	6.9	7.4	7.7	8.5	7.6	3.5	1.11
Average		40	5.0	5.1	5.0	5.5	5.2	2.2	1.20
1931	Silver	50	7.0	8.9	11.8	12.4	10.0	5.8	.88
	Light green	50	5.3	5.6	6.9	7.7	6.4	3.5	.93
1932	Tree-ripe	50	7.3	7.5	8.2	8.7	7.9	3.5	1.15
1933	Green	50	17.9	17.4	16.6	14.8	16.7	8.1	1.05
	Silver	50	14.6	14.5	14.3	15.8	14.8	6.9	1.09
Average		50	10.4	10.8	11.6	11.9	11.2	5.6	1.02
1931	Silver	60	12.7	13.5	13.9	14.0	13.5	7.0	.98
	Light green	60	8.9	10.4	11.2	11.6	10.5	6.0	.89
1932	Tree-ripe	60	14.5	14.3	13.3	13.9	14.0	6.7	1.07
1933	Green	60	28.6	24.5	20.0	16.8	22.5	11.3	1.02
	Silver	60	22.1	19.8	17.2	15.5	18.6	8.4	1.13
Average		60	17.4	16.5	15.1	14.4	15.8	7.9	1.02
1931	Silver	70	18.3	19.4	18.1	17.8	18.4	10.0	.94
	Light green	70	21.8	23.2	24.7	24.4	23.5	12.3	.97
1932	Tree-ripe	70	23.8	22.9	23.7	22.9	23.3	11.6	1.02
1933	Green	70	34.8	29.1	19.8	17.6	25.3	14.7	.88
	Silver	70	29.4	19.7	22.1	20.1	22.8	12.4	.94
Average		70	25.6	22.9	21.7	20.6	22.7	12.2	.95
1931	Silver	80	30.5	30.7	26.5	24.9	28.2	15.5	.93
	Light green	80	24.7	21.1	19.6	16.9	20.6	12.7	.83
1932	Tree-ripe	80	24.6	21.0	19.1	19.1	20.9	8.3	1.28
1933	Green	80	40.0	30.7	25.3	21.8	29.5	15.6	.96
	Silver	80	38.0	29.2	25.2	22.5	28.7	13.2	1.11
Average		80	31.6	26.5	23.1	21.0	25.6	13.1	1.02
1931	Silver	90	49.7	41.4	37.5	34.6	40.8	17.4	1.20
	Light green	90	62.4	52.7	50.8	50.1	54.0	23.9	1.15
1932	Tree-ripe	90	36.6	31.4	28.5	27.5	31.0	14.4	1.10
1933	Green	90	50.0	35.6	33.5	28.7	37.0	16.3	1.16
	Silver	90	52.3	35.0	33.5	32.6	38.3	15.2	1.28
Average		90	50.2	39.2	36.8	34.7	40.2	17.4	1.18
1931	Silver	100	71.9	78.5	85.6	85.1	80.3	30.0	1.36
	Light green	100	91.7	87.5	89.9	90.1	89.8	36.3	1.26
1932	Tree-ripe	100	63.7	66.3	74.1	81.1	71.3	24.2	1.50
Average		100	75.8	77.4	83.2	85.4	80.5	30.2	1.37

[1] Runs were for 1 day, except at 32° and 40° F., at which they were for 2 days.

In the 1933 season the lemons respired at a much higher average rate at 32° to 60° F. than in the two previous seasons (table 3). In some instances at the lower temperatures, the rate in 1933 was at least double the rates of the previous seasons. On the other hand, at 90° the rates in 1931 were for the most part higher than those in 1933. The reasons for these differences are not known, but probably they are due to some cultural or climatic factor.

Curves for the average rates at the different temperatures are shown in figure 4, *E*. Contrary to the results with the other citrus fruits, the rate of respiration of lemons at 100° F. increased with time. As with the other fruits, the rate decreased with time at 90° to 60°. At 50° there was a slight increase, and at 40° and 32° there was little or no change during the period studied. With increase in temperature there was a steady increase in respiratory rate, the increases in carbon dioxide and oxygen being closely parallel up to 80°. Above 80° the rates increased more rapidly, particularly in carbon dioxide (table 3).

The respiratory ratios approximated 1.0 between 50° and 80° F. (table 3 and fig. 2, *G*), with somewhat higher ratios above and below these points. As indicated previously, a ratio of 1.0 indicates the respiration of sugar. It is rather surprising to find a fruit with such a high acid content apparently respiring only sugar over such a wide range of temperatures. The respiratory ratio at 100° averaged only slightly above 1.33, indicating little or no intramolecular respiration at this temperature in lemons, although considerable intramolecular respiration was indicated in the other citrus fruits at this temperature. The smaller size of the lemon fruit, and consequently the greater surface area per unit of weight, may be responsible for the lack of intramolecular respiration, because the greater surface area would facilitate gaseous exchange.

TEMPERATURE COEFFICIENTS

Van't Hoff's temperature coefficient (Q_{10}) expresses the rate of increase in the velocity of a reaction with each increase in temperature of 10° C. (18° F.). Temperature coefficients of 2.0 to 3.0 or more are typical of chemical reactions, and Mathews (*12*) stated that between 10° and 40° C. (50° and 104° F.) nearly all vital activities are doubled or trebled by a rise in temperature of 10° C. A Q_{10} of less than 2.0 is more typical of a physical process. The Q_{10} of a chemical reaction is generally higher at low temperatures. The temperature coefficients for the respiration (carbon dioxide output and oxygen intake) of citrus fruits are shown for overlapping ranges (18° F.) of temperature in table 4 for the early results, in which a wider total range of temperatures was used, and in table 5 for later results of carbon dioxide evolved at 32° to 70°. At the lowest temperature range (32° to 50°) the rate generally increased more than three times (average 3.9 and 3.6 in the early results and 5.1 in the later results). The Q_{10} decreased with increased temperature and reached a minimum at 62° to 80° or 72° to 90°, with average minima of 1.5. At the higher temperatures (82° to 100° and 92° to 110°) the Q_{10} increased. The lower coefficients between 62° and 90°, particularly for oxygen intake, indicate that physical processes, possibly diffusion of the gases, control the rate of oxidation at this range of temperatures.

Trout et al. (*19*) indicated for oranges a Q_{10} of 4.0 from 36° to 59° and of 1.5 from 59° to 78°. These values are in good agreement with those shown in tables 4 and 5. Contrary to the results presented herein, Trout et al. reported a further decrease in Q_{10} to 1.0 between 78° and 90°.

TABLE 4.—*Temperature coefficients (Q_{10}) of respiration (carbon dioxide output and oxygen intake) of citrus fruits, 1931–32*

Temperature range (° F.)	Oranges						Grapefruit				Lemons		Average		
	Florida		California				Florida		California (Marsh)		California (Eureka)				
			Washington Navel		Valencia										
	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2 and O_2
	Q_{10}	Q_{10}	Q_{10}	Q_{10}	Q_{10}	Q_{10}	Q_{10}	Q_{10}	Q_{10}	Q_{10}	Q_{10}	Q_{10}	Q_{10}	Q_{10}	Q_{10}
32 to 50	3.9	2.8	3.3	4.2	6.0	5.2	2.7	2.0	4.0	3.7	3.7	3.7	3.9	3.6	3.8
42 to 60	2.8	2.7	2.9	3.4	2.1	2.2	2.6	2.3	2.4	2.1	2.5	2.8	2.5	2.6	2.6
52 to 70	2.2	2.2	1.8	1.8	1.5	1.8	2.2	1.9	1.9	2.3	1.9	2.1	1.9	2.0	2.0
62 to 80	1.6	1.8	1.5	1.2	1.6	1.6	1.4	1.2	1.7	1.7	1.5	1.2	1.5	1.5	1.5
72 to 90	1.5	1.1	1.6	1.3	1.4	1.3	1.6	1.3	1.3	1.2	1.7	1.4	1.5	1.3	1.4
82 to 100	1.8	1.2	2.7	1.6	2.0	1.4	2.3	1.8	2.3	1.3	1.9	2.2	2.2	1.6	1.9
92 to 110	2.0	2.0	------	-----	------	-----	2.2	2.7	------	-----	------	-----	2.1	2.3	2.2

HEAT OF RESPIRATION

In the respiration process, energy is released principally in the form of heat. The heat of respiration is generally computed from the carbon dioxide evolved, on the assumption that this is derived from the complete oxidation of a hexose sugar and that all the energy is released in the form of heat. It will be pointed out later that reactions other than the complete oxidation of sugar may and probably do occur and that the oxygen reading should be a more reliable measure of the energy or heat released.

The heat of respiration in British thermal units (B. t. u.) per ton of fruit per day is presented in tables 6 and 7. The heat of respiration was computed from the carbon dioxide evolved by multiplying the milligrams of carbon dioxide evolved per kilogram of fruit per hour by the factor 220. For the earlier results, in which a greater range of temperatures was used, the heat of respiration in British thermal units per ton-day was computed from the oxygen intake as well, by multiplying the cubic centimeters of oxygen intake per kilogram of fruit per hour by the factor 423.65. Thus the heat of respiration was directly proportional to the rate of respiration and varied as the respiratory rate varied. For oranges, as computed from CO_2 evolved, the heat of respiration ranged from 427 to 979 B. t. u. at 32° F., and increased to 22,660 B. t. u. at 100°. For lemons, the heat of respiration, similarly computed, ranged from an average of 675 B. t. u. at 32° to 17,710 at 100°. For grapefruit, the heat of respiration, similarly computed, ranged from 385 to 528 B. t. u. at 32° and increased to over 11,000 B. t. u. at 100°. At 32° to 80°, the average heat of respiration, when computed from the carbon dioxide determinations, was 14 to 27 percent higher than when computed from the oxygen determinations, and above 80° the difference was even greater (40 to 96 percent).

TABLE 5.—*Temperature coefficients* (Q_{10}) *of respiration (carbon dioxide output) of citrus fruits, 1941-42*

Temperature range (° F.)	Oranges													Grapefruit, Florida						
	Florida									California			Marsh [1]		Duncan [1]		Foster	Thompson	Average
	Washington Navel	Parson Brown		Valencia						Washington Navel	Valencia		a	b	a	b			
		Sour orange	Rough lemon	Early picking		Midseason picking		Late picking			Early picking	Late picking							
				Sour orange	Rough lemon	Sour orange	Rough lemon	Sour orange	Rough lemon										
	Q_{10}	Q_{10}	Q_{10}	Q_{10}	Q_{10}	Q_{10}	Q_{10}	Q_{10}	Q_{10}	Q_{10}	Q_{10}	Q_{10}	Q_{10}	Q_{10}	Q_{10}	Q_{10}	Q_{10}	Q_{10}	Q_{10}
32 to 50	6.3	7.1	6.4	4.7	5.5	5.7	5.7	5.1	5.5	5.2	5.4	4.3	4.0	4.2	4.7	3.8	4.8	4.0	5.1
42 to 60	2.4	2.8	2.4	2.3	2.3	2.6	2.6	2.5	2.5	2.2	2.1	2.1	2.0	2.7	2.2	2.5	2.9	2.7	2.4
52 to 70	2.0	1.8	2.0	1.8	1.8	2.0	2.0	1.9	1.9	1.7	1.5	1.9	1.6	2.2	1.6	2.4	2.3	2.3	1.9

[1] a=Fruit from Vero Beach section on sour orange rootstock; b=fruit from Davenport section on rough lemon rootstock.

TABLE 6.—*Heat of respiration of citrus fruits at different temperatures, computed from respiratory activity determinations of 1929 to 1933*

[Expressed as B. t. u. per ton of fruit per day]

Temperature (°F.)	Oranges						Grapefruit				Lemons		Average of ratios $\left(\frac{a}{b}\right)$
	Florida		California				Florida		California, Marsh		California, Eureka		
			Washington Navel		Valencia								
	a[1]	b[2]	a[1]	b[2]	a[1]	b[2]	a[1]	b[2]	a[1]	b[2]	a[1]	b[2]	
	B.t.u.	B.t.u.	B.t.u.	B.t.u.	B.t.u.	B.t.u.	B.t.u.	B.t.u.	B.t.u.	B.t.u.	B.t.u.	B.t.u	
32	686	721	895	621	427	382	455	379	499	390	675	633	1.18
40	1,410	1,204	1,397	1,124	1,034	864	1,069	935	845	759	1,142	915	1.19
50	2,706	2,131	2,970	2,618	2,589	1,972	1,522	1,175	1,980	1.368	2,508	2,332	1.26
60	4,554	3,692	4,994	4,656	2,750	2,400	2,772	2,244	2,552	1,942	3,476	3,297	1.18
70	6,578	5,382	6,006	5,244	3,927	3,566	3,520	2,718	3,872	3,448	4,994	5,118	1.14
80	7,788	5,462	8,008	5,789	4,554	4,124	4,180	2,785	4,818	3,973	5,654	5,495	1.27
90	10,230	6,116	10,428	7,132	5,720	4,682	6,050	3,692	5,170	4,380	8,844	7.299	1.40
100	15,202	7,379	22,660	9,858	9,913	5,814	11,308	5,537	11,396	5,139	17,710	12,669	1.96
110	22,242	12,442	------	------	------	------	17,952	10,907	------	------	------	------	1.72

[1] Computed from CO_2 measurements (220 × mg. CO_2 per kilogram-hour), assuming complete oxidation of hexose sugar (glucose).

[2] Computed from O_2 measurements (423.65 × cc. O_2 per kilogram-hour), assuming complete oxidation of both hexose sugars (glucose and fructose) and citric acid.

TABLE 7.—*Heat of respiration of citrus fruits at 32°, 50°, and 70° F., based on 1941–42 determinations of carbon dioxide evolved*

[Expressed as B. t. u. per ton of fruit per day]

Temperature (°F.)	Oranges					Grapefruit			
	Florida			California		Florida			
	Washington Navel[1]	Parson Brown[2]	Valencia[3]	Washington Navel	Valencia[4]	Marsh[5]	Duncan[5]	Foster	Thompson
	B.t.u.	B.t.u.	B.t.u.	B.t.u.	B.t.u.	B.t.u.	B.t.u.	B.t.u.	B.t.u.
32	616–660	440–473	451–979	858	495–627	407–440	385–396	407	528
50	3,938–4,070	2,805–3,366	2,464–4,598	4,334	2,552–2,706	1,716–1,760	1,463–1,859	1,947	2,090
70	8,360–9,020	5,707–6,732	5,313–9,119	7,755	4,048–5,896	2,937–4,257	3,366–3,916	5,423	5,621

[1] Range in B. t. u. values due to location of trees. (See table 1, lot 7.)

[2] Range in B. t. u. values due to rootstock (lower value for rough lemon and higher value for sour orange). (See table 1, lot 8.)

[3] Range in B. t. u. values due to rootstock and maturity (lower value for rough lemon picked late and higher value for sour orange picked early). (See table 1, lots 9, 10, and 11.)

[4] Range in B. t. u. values due to maturity. (See fig. 3, *D*.)

[5] Range in B. t. u. values due to rootstock and soil type. (See table 2.)

EFFECT OF ETHYLENE TREATMENTS ON RESPIRATION

METHODS

Ethylene, which is used for the degreening of citrus fruits, is usually applied at the rate of 1 part to 5,000 of air in intermittent charges or 1 part to 50,000 in continuous flow for 48 to 60 hours at 80° to 85° F. (*20*). In addition to destroying chlorophyll, ethylene greatly stimulates the respiratory activity of citrus (*1; 2; 3; 9; 17, pp. 42–44*)[4] and other fruits. It seems likely that the effect of ethylene on respiratory rate may be a measure also of the effect of ethylene on the rate of degreening.

In order to study the effect of ethylene on respiration, the gas was added to the respiration chambers after the fruit was sealed in the

[4] See also footnote 3, p. 334.

chambers.   This was done by removing the funnel from the upper inlet tube and displacing the desired volume of ethylene into the tube and washing it into the chamber with water.   In most instances the desired
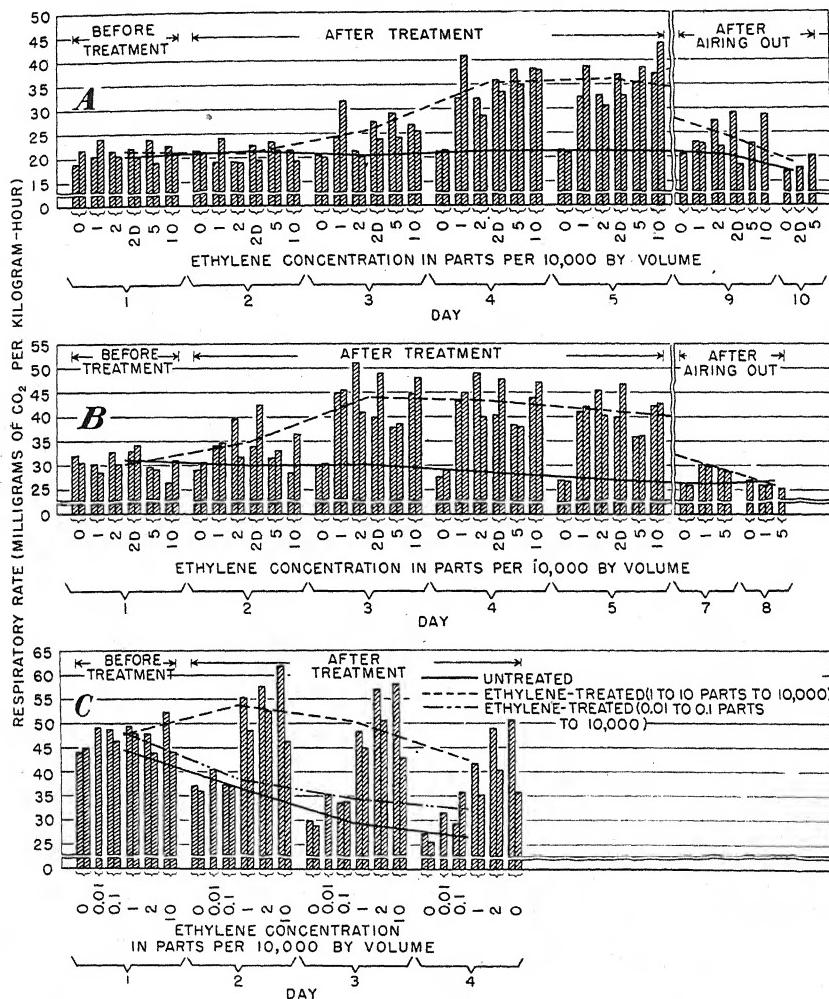
FIGURE 5.—Effect of ethylene treatments on respiratory activity of Florida-grown oranges at 60° F. (*A*), 70° (*B*), and 85° (*C*).   The ethylene treatments were applied only once except for those marked 2D, in which successive applications were made daily for 4 days.

quantity of ethylene was introduced only once.   In a few instances daily doses of ethylene were added for several days.

FRUITS TESTED

ORANGES

Figure 5 shows the results of a study of the effects of ethylene on the respiration of Florida oranges at 60°, 70°, and 85° F.   The fruit

tested at 60° was shipped from Frostproof, Fla., on March 18, 1930, and was received 4 days later. It had not been treated previously with ethylene. Only four oranges were used in each respiration chamber, but the respiratory activity was determined on duplicate lots. Ethylene was not added during the first 2 days' runs, and the results for these 2 days (fig. 5, *A*) show no significant difference in the respiratory rates of the control lots and those that were to be treated with ethylene. At the end of the second day's run ethylene was added to the different chambers at the rate of 0, 1, 2, 5, and 10 parts to 10,000 parts of air by volume. Ethylene (2 parts to 10,000) was added to two lots after the third and fourth days' runs as well as after the second day's run. The daily additions of ethylene did not increase the respiratory rates any more than did the single application after the second run; nor was there any significant difference in the increased respiratory rates that could be attributed to differences in the concentration of ethylene from 1 to 10 parts per 10,000 parts of air. For this reason it seemed permissible to plot the respiratory curve for the average of all concentrations used in the ethylene treatments. This curve shows an increase of about 25 percent in respiratory activity, as compared with that of the controls, during the first day after the ethylene additions. The maximum rates were attained on the second and third days after the ethylene additions, when an average increase of about 70 percent above the controls was shown. After 2 days in which the respiration chambers were opened for airing out, the respiratory rate of the ethylene-treated lots dropped to approximately that of the control lots.

Similar results at 70° F. are shown in figure 5, *B*, for Valencia oranges shipped from Bradenton, Fla., on February 20, 1930. The determinations of respiratory rate were started on March 10. In the first run (before the ethylene was added) the respiration of lots to be treated did not differ significantly from that of the control lots. As at 60° (fig. 5, *A*), the different concentrations of ethylene or the daily addition of ethylene at 70° did not result in any significant difference in the respiratory response, and the results from all ethylene treatments were averaged. During the first day, this average response to ethylene showed a slight increase (about 15 percent) over the controls. The maximum response was attained the second day, with an average increase of about 47 percent in respiratory activity. Thereafter the respiratory rate of the ethylene-treated lots showed a gradual decrease, and even in the lots that were given daily doses of ethylene there was a corresponding decrease in rate. After the chambers were aired out for a day, the respiratory rate of the ethylene-treated lots dropped to approximately that of the controls and subsequently even below the controls.

The results of using ethylene at 85° F are shown in figure 5, *C*, for oranges shipped directly from Florida. The determinations were made May 12 to 16, 1930. In addition to concentrations of 1, 2, and 10 parts per 10,000 by volume, lower concentrations of 1 to 100,000 and 1 to 1,000,000 were used. During the first run, before the application of the differential treatments, the respiration of the control lots averaged somewhat lower than that of the lots to be treated. The untreated samples showed a rather rapid decrease in respiratory rate from nearly 45 mg. of carbon dioxide per kilogram-hour on the first day to about 26.5 mg. on the fourth day. The lots treated with the

lower ethylene concentrations (1 to 100,000 and 1 to 1,000,000) averaged somewhat higher in respiratory rate than the controls before treatment but showed a similar decrease in rate with time, indicating that such low concentrations of ethylene had no effect on the respiratory activity. Samples treated with the higher concentrations (1, 2, and 10 parts per 10,000) of ethylene showed a marked increase in respiratory rate during the first day after treatment, with a rather rapid decline thereafter but with the rate maintained considerably above that of the control lots.

Washington Navel oranges from San Dimas, Calif., were picked and shipped on March 6, and respiration determinations were started
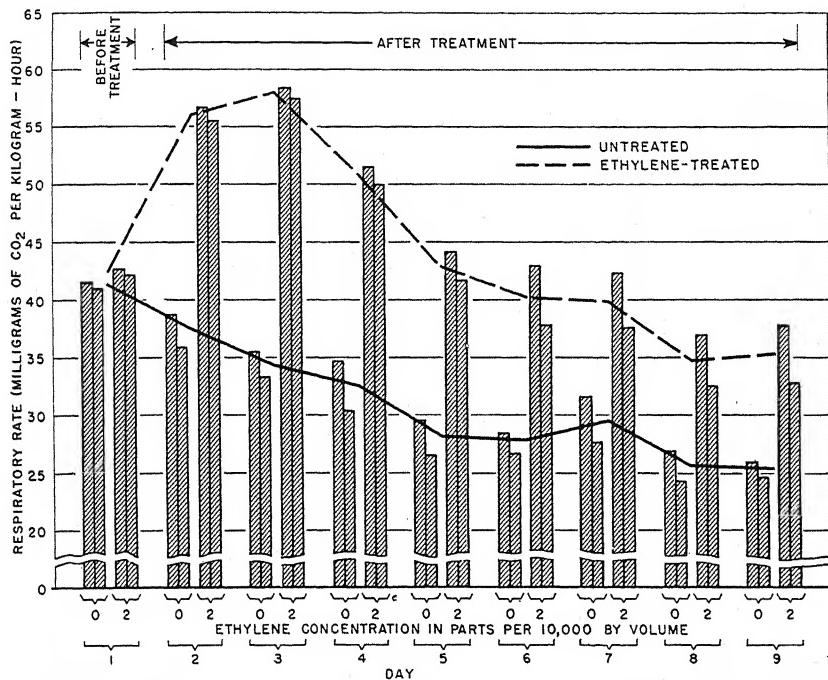
FIGURE 6.—Effect of ethylene treatments on respiratory activity at 80° F. of Washington Navel oranges grown in California.

March 12. Three oranges were tested at 80° F. in each respiration chamber and, as usual, determinations were made on duplicate lots. One concentration only (2 to 10,000) of ethylene was applied after the first run. The results are shown in figure 6.

During the first run the lots to be treated with ethylene averaged slightly higher in respiratory rate than the control lots. The control lots showed a rather rapid decrease in rate from 41.3 mg. of carbon dioxide per kilogram-hour on the first day to 25.3 mg. on the ninth day. The ethylene-treated lots showed a marked increase in respiratory activity during the first day after the introduction of the ethylene, and averaged 50 percent greater than the control lots. There was a further increase in the respiratory rates of the treated lots during the second day after treatment, when these lots averaged 69 percent

greater than the controls. A sharp decline in the respiratory rate of the treated lots followed, but on the seventh day after treatment these lots averaged 36 percent higher in respiratory rate than the untreated lots.

GRAPEFRUIT

As with oranges, the respiratory activity of grapefruit as influenced by treatments with ethylene was determined at 60°, 70°, and 85° F.
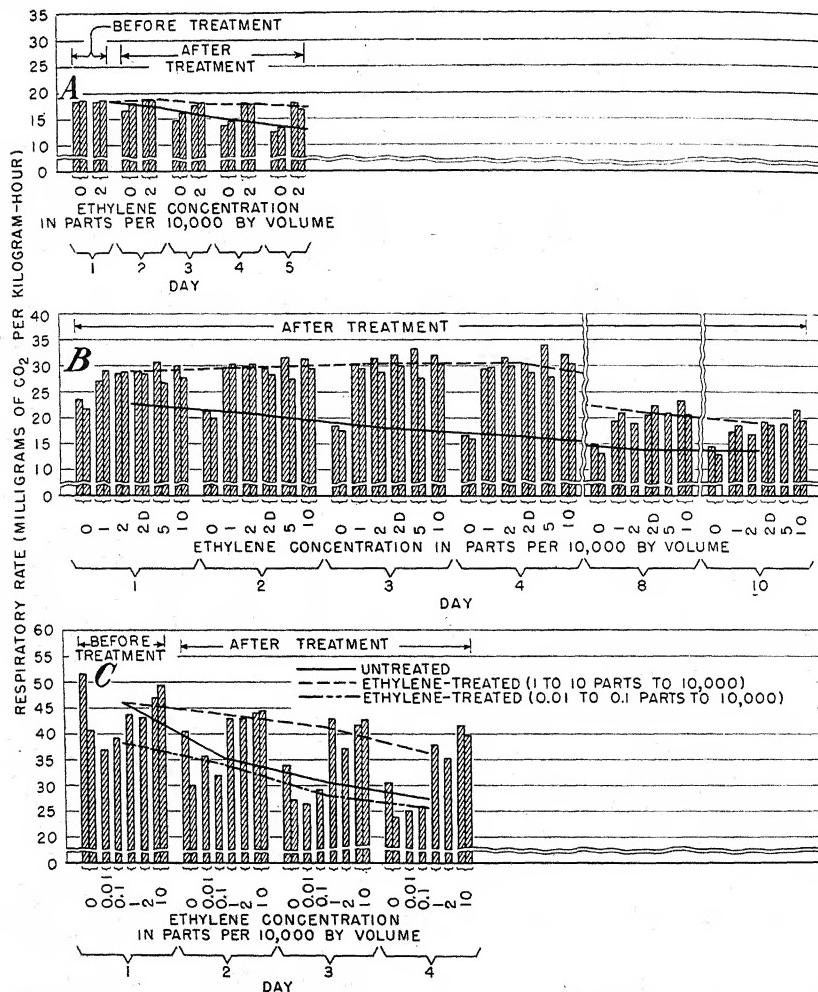
FIGURE 7.—Effect of ethylene treatments on respiratory activity of Florida-grown grapefruit at 60° F. (*A*), 70° (*B*), and 85° (*C*). The ethylene treatments were applied only once except for those marked 2D, in which successive applications were made daily for 4 days.

The fruit tested at 60° was shipped from Orlando, Fla., and had been waxed by the Brogdex process. It was purchased on the Washington, D. C., market on January 10, 1930. Results of the ethylene treatments on this fruit are shown in figure 7, *A*. The untreated fruit

(without ethylene) showed a gradual decline in respiratory activity from 18.4 to 12.8 mg. of carbon dioxide per kilogram-hour, whereas the respiratory activity of the treated fruit remained nearly constant and was about 36 percent higher than that of the untreated fruit by the fourth day after treatment. In commercial practice the ethylene treatment would be applied before the waxing, so these results may not be representative of commercial conditions.

The fruit tested at 70° F. was shipped directly from Arcadia, Fla., on February 14, and the determinations were started on February 24, 1930. As with oranges, there was no significant difference between the different concentrations of ethylene (within the limits of 1 to 10 parts per 10,000) in their effects on the respiratory rates (fig. 7, *B*). The daily addition of ethylene for 4 days did not increase the rate more than a single addition at the beginning of the experiment. Consequently, results with all of the ethylene-treated fruit were averaged for comparison with those from fruit receiving no ethylene. The untreated lots showed a gradual decline in respiratory activity from 22.5 mg. of carbon dioxide per kilogram-hour on the first day to 16.3 mg. on the fourth day. The respiratory activity of the ethylene-treated lots averaged 26.5 percent greater on the first day and reached a maximum increase on the fourth day of 87 percent, with a respiratory activity of 30.5 mg. of carbon dioxide per kilogram-hour. After several days in which the fruit was aired out, the rate of respiration of the treated lots decreased greatly but was still about 37 percent greater than that of the controls 10 days after the ethylene was added and 6 days after the fruit was aired out.

The fruit tested at 85° F. was shipped directly from Florida on February 20, 1930, and had not been treated with ethylene. It was held at 32° until the beginning of the experiment on May 5, 1930. The results (fig. 7, *C*) show a rapid decrease in the respiratory rate of the untreated lots from an average of 46 mg. of carbon dioxide per kilogram-hour on the first day to 27.2 mg. on the fourth day. The lots that were treated with the lower concentrations of ethylene (1 to 100,000 and 1 to 1,000,000) averaged lower in respiratory rate than the control lots on the first day (before they were treated). Although they did not decrease in rate as greatly as the controls on the first day after treatment, the difference in rate was not significant. The lots treated with the higher concentrations (1, 2, and 10 parts per 10,000) of ethylene averaged the same as the control lots before the treatments. After treatment their respiratory rates decreased but more slowly than those of the controls, and averaged 24 percent higher on the first day after treatment and 42 percent higher on the third day after treatment.

LEMONS

The respiratory rate of Eureka lemons at 80° F., as influenced by ethylene, is shown in figure 8. The dark- and light-green lemons were shipped from San Dimas, Calif., by nonrefrigerated express on April 10 and received on April 15; the respiration determinations were started on April 16, 1931. The silver lemons were shipped by express from San Dimas on May 8, 1931, and received May 13; the determinations were started May 18. As the silver lemons were picked at a much later date, they were not directly comparable with the less mature lemons but showed a similar response to the ethylene treatment.

Therefore, results obtained with fruit of all three stages of maturity were averaged to determine the influence of ethylene on respiration.

In the first run (before the ethylene was added) the lots to be treated averaged somewhat higher in respiratory activity (15 percent) than the control lots. The lemons showed a greater response to ethylene treatment than either oranges or grapefruit. The respiratory
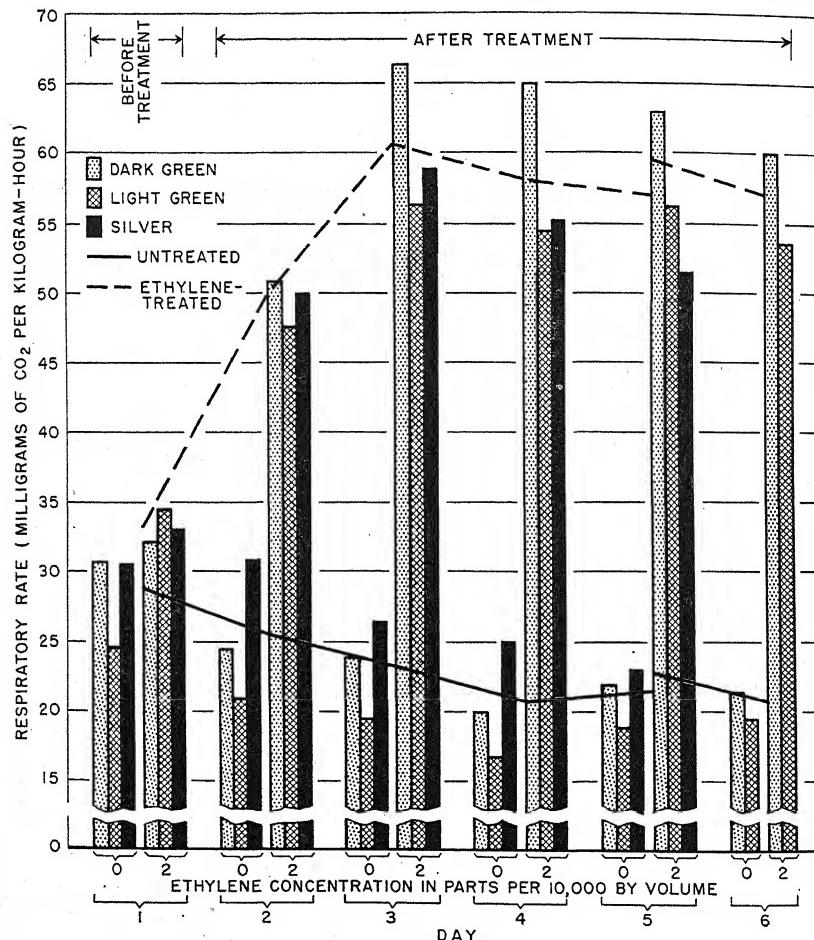
FIGURE 8.—Effect of maturity and ethylene treatments on respiratory activity at 80° F. of Eureka lemons grown in California.

rates of the treated lemons increased from 33.2 mg. of carbon dioxide per kilogram-hour before treatment to 49.8 mg. on the first day after treatment and reached a peak of 60.7 mg. on the second day after treatment. This represented an increase over the untreated lots of 96 percent the first day after treatment and 160 percent the second day. Five days after treatment with ethylene, the treated lots (green lots only) were respiring at a rate 178 percent higher than that of the control lots.

EFFECT OF DECAY ON RESPIRATION OF LEMONS

In a number of instances the respiratory activity of certain lots of fruit increased greatly and was out of line with the previous trend. An example of this is shown in figure 1, *C*, at 70° F. An examination of such lots generally disclosed the presence of decay in one or more of the fruits, and such results were usually discarded. In one instance slight mold on a single fruit apparently resulted in large increases in the carbon dioxide evolved. In order to determine the
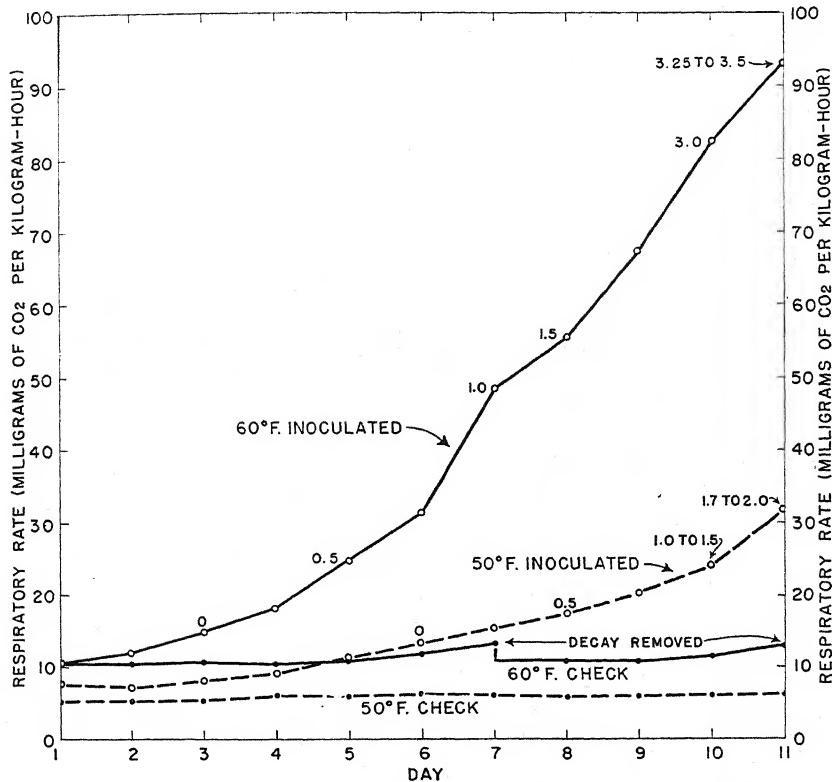
FIGURE 9.—Effect of inoculations with *Penicillium digitatum*, causing green mold decay, on respiratory activity at 60° and 50° F. of California-grown Eureka lemons, 1931. Numbers on curves represent estimated diameter (inches) of lesions.

extent to which decay might increase the carbon dioxide evolved, lemons were inoculated with cultures of green mold (*Penicillium digitatum* Sacc.) and the black rot fungus (*Alternaria citri* Ell. and Pierce). The lemons for the first experiment were shipped from California on July 2, and the experiment was started on July 15, 1931. The respiratory activity of the fruit was determined at 40°, 50°, and 60°.

The results of the experiment are shown in figure 9. During the course of the experiment no decay developed at 40°F, and therefore no data for 40° are presented in the figure. *Alternaria citri* did not

develop at any of the temperatures, and the results for this fungus also are omitted. At 50° (fig. 9) the respiratory activity of the inoculated fruit started off considerably higher than that of the untreated checks. After 6 days the inoculated lemons were respiring at double the rate of the controls, though no evidence of decay lesions was apparent. By the eleventh day decay lesions about 1.75 to 2 inches in diameter had developed, and the carbon dioxide evolved by the decaying lemons was over five times that of the controls.

At 60° F., after 11 days the decay lesions were 3.25 to 3.5 inches in diameter and the respiratory rate was 93 mg. of carbon dioxide per kilogram-hour. The sound lemons generally respired at the rate of
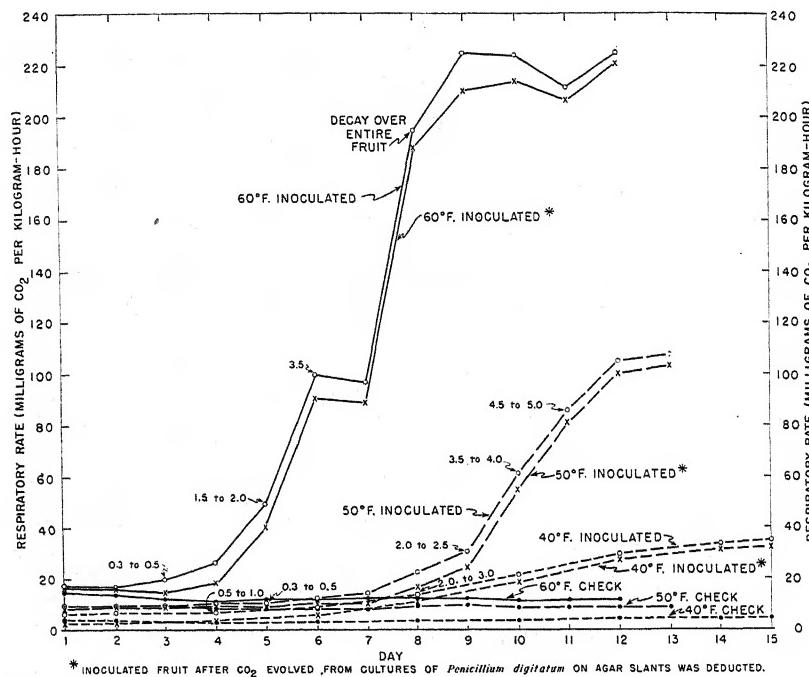
FIGURE 10.—Effect of inoculations with *Penicillium digitatum* on respiratory activity at 60°, 50°, and 40° F. of California-grown Eureka lemons, 1932. Numbers on curves represent estimated diameter (inches) of lesions.

about 11 mg. of carbon dioxide per kilogram-hour. However, slight decay developed in the check fruit on two occasions and increased the respiratory activity somewhat. The highest rate attained by the inoculated fruit at 60° was more than eight times the average rate of the control fruit. No doubt a considerably higher rate would have been attained by the inoculated fruit if the experiment had continued, because the trend was still steeply upward when the test was terminated.

A second experiment to determine the effect of decay on respiration was made on full-ripe lemons from San Dimas, Calif., received April 23, 1932, and held at 40° F. until the beginning of the experiment on May 4, 1932. As decay had not developed at 40° in the first experi-

ment, the inoculated lemons in the second experiment were incubated for 3 days at 60° before being transferred to 40°. To determine whether wounding would exert a stimulating effect on respiration, one each of the duplicate uninoculated lots of fruit at 50° and 60° was punctured with a sterile needle; thus the uninoculated fruit was wounded in the same manner as the inoculated fruit. The wounding had no apparent effect on respiration, and the curves in figures 9 and 10 are based on averages of the duplicate determinations.

The first experiment had not shown what part of the stimulation in respiratory activity of the decaying fruit was due to the fruit and what part to the decay organism; so in the second experiment an equal number of potato-dextrose agar slants in test tubes were inoculated at the same time that the lemons were inoculated and the carbon dioxide evolved by the cultures was determined separately (fig. 10). This was deducted from the total carbon dioxide evolved by the decaying lemons to get the net effect of the decay on the respiratory activity of the lemons. It is recognized that the lemon fruit may supply a more or a less favorable medium for the organism so that it may grow and respire more or less rapidly on the fruit than on the culture medium; hence the carbon dioxide evolved on the culture medium may only approximate that given off by the organism growing on the fruit.

The results, presented in figure 10, show a stimulation of carbon dioxide evolution similar to that shown in figure 9. On the ninth day after inoculation the respiratory activity of the decaying fruit at 60° F. reached a maximum of over 220 mg. of carbon dioxide per kilogram-hour, as compared with only about 12 mg. for the sound fruit. After the carbon dioxide evolved by the agar cultures was deducted, the respiratory rate of the inoculated fruit reached a maximum of over 210 mg. of carbon dioxide, or more than 17 times that of the controls. At 50° the respiratory activity of the inoculated lemons did not begin to increase sharply until 7 to 8 days after inoculation, whereas at 60° there was an increase after 3 to 4 days. The inoculated fruit at 50° reached a respiratory rate of over 100 mg. of carbon dioxide per kilogram-hour by the twelfth and thirteenth days and this was about the maximum. At both 50° and 60° the maximum respiratory activity of the decaying fruit was attained at about the time that decay had completely covered the surface of the fruit. The maximum respiratory activity attained at 50° by the inoculated fruit was about 12 times that of the sound fruit. At 40° the decaying lemons showed a more gradual increase in carbon dioxide evolved than at the higher temperatures. After 16 days the rate of carbon dioxide evolution of the inoculated fruit was still increasing, so that the maximum probably had not been attained. At this time, about 75 percent of the surface showed decay and the respiratory rate was over 8 times that of the control fruit.

The results shown in figure 10 indicate that the carbon dioxide evolved by the decay organism was responsible for a relatively small percentage of the increase in carbon dioxide evolved by decaying lemons. Recently Biale (1) has shown that the vapor from lemons decaying because of *Penicillium digitatum* stimulated the respiratory activity of sound lemons exposed to the vapor from the decaying lemons. The stimulation amounted to 50 to 100 percent and was

attributed to the evolution of ethylene by the decay organism. Miller et al. (*15*) showed that various citrus fruits, including lemons, produce epinasty in test plants, indicating the evolution of ethylene. They showed further that decaying fruit inoculated with *P. digitatum* produced epinasty more rapidly than sound fruit and that agar cultures of *P. digitatum* also produced epinasty. Biale (*1*) reported that vapor from other decay organisms (*P. italicum, Sclerotinia sclerotium,* and *Aspergillus niger*) had no stimulatory effects on respiration. Since the maximum stimulatory effect of ethylene on lemons was less than 200 percent (fig. 8), this would account for only a fraction of the increased respiratory activity observed in decaying lemons (figs. 9 and 10). It seems likely that the disorganization of the tissue resulting from decay would tend to greatly increase the activity of the hydrolytic and catabolic processes, and this may account in large measure for the great increase in carbon dioxide evolved. The observed increase in respiration of lemons is much greater than the increase of 100 percent observed by Kidd (*10*) in apples.

RESPIRATION OF FLESH OF PEELED LEMONS

The rind of citrus fruits, particularly that of lemons, represents a rather large proportion of the total weight of the fruit. Kudryavzeva (*11*) observed that the sugar and acid remained fairly constant in the flesh of mandarin oranges during storage but that there was considerable dry matter lost from the rind. He suggested that in respiration only the materials in the rind were used. Trout et al. (*19*) also observed that the respiration of oranges was more closely related to the composition of the rind than to that of the juice. Data presented by Miller and Schomer (*14*) show the percentage of total sugar in the rind of lemons to be double that in the flesh, with more lost from the rind than from the flesh during storage. These analyses indicate that in determinations of respiratory activity of citrus fruits the results are influenced more by physiological reactions in the rind than in the flesh.

It was found that with care lemons could be peeled so as to leave a very thin layer of albedo, without injury to the flesh. When exposed to the fairly dry atmosphere of the laboratory the thin layer of albedo dried and formed a thin parchmentlike skin on the lemon, with no apparent wilting of the flesh. Lemons peeled in this manner were used to determine the respiratory activity of the flesh alone. The results in table 8 show that the rind constituted about a third of the total weight of the fruit. In order to maintain a low relative humidity, and thus retard decay on the peeled fruit, concentrated sulfuric acid was placed in the bottom of the respiration chambers. In spite of this precaution, 60 percent of the peeled fruit showed decay (mostly slight) when removed after 5 days in the chambers. For this reason the determinations for only 3 days were used, as there was a large increase in respiratory activity in the peeled fruit on the fourth and fifth days, apparently because of the decay. The data in table 8 indicate that the flesh respires in proportion to its weight at practically the same rate as the whole fruit. However, the evidence is not conclusive, as there may have been some stimulatory effect of the rather severe wounding on the respiratory rate of the peeled lemons.

TABLE 8.—*Respiratory activity of whole and peeled lemons*

[Expressed as milligrams of CO_2 per kilogram-hour]

Treatment	Total lemons	Weight		CO_2 evolved			
		With peel	Without peel	First day	Second day	Third day	Average
	Number	*Grams*	*Grams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
Not peeled	15	1,630.5		17.2	13.6	12.3	14 4
Peeled	15	1,586.5	1,071.0	16.1	14.2	13.8	14.7

EFFECT OF ARSENATE SPRAY ON RESPIRATION OF GRAPEFRUIT

Citrus fruit from trees sprayed with arsenicals has been found to be lower in acid content than fruit from trees not so sprayed. Since arsenical sprays apparently have a rather marked physiological effect on the fruit it was desirable to study its effect on respiration. For this purpose Marsh and Duncan grapefruits that had been sprayed with lead arsenate (1 pound to 100 gallons) were shipped from Florida on April 8, 1943, and the respiratory activity was determined at 70° F. There was no appreciable difference in the amount of soluble solids in the juice between fruit from sprayed trees and that from unsprayed trees. Spraying reduced the acidity from 1.21 to 1.14 gm. per 100 ml. of juice in the Marsh variety and from 1.55 to 1.38 gm. per 100 ml. in the Duncan variety. The effect of spraying on the respiratory activity of the fruit at 70° is shown in table 9. Spraying reduced the average respiratory activity of both varieties. The reduction was only about 8 percent for Marsh but was nearly 20 percent for Duncan. The effect of spraying with arsenicals appears to be to lower the metabolic activity of the fruit as measured by respiratory rate. This is associated with lower acidity of the juice.

TABLE 9.—*Effect of spraying trees with lead arsenate (1 pound to 100 gallons) on the respiratory activity at 70° F. of grapefruit grown in Florida*

[Expressed as milligrams of CO_2 per kilogram-hour]

Variety	Treatment	CO_2 evolved in lot—				Average
		1	2	3	4	
		Milligrams	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
Marsh	Not sprayed	19.6		20.0	23.6	21.1
	Sprayed	17.6	15.9	23.9	20.8	19.5
Duncan	Not sprayed	19.3	23.5	19.4	19.5	20.4
	Sprayed	15.8	15.2	16.8	18.0	16.5

DISCUSSION

The results of the study of the effect of ethylene on the respiration of oranges, grapefruit, and lemons are in general agreement with those reported by others in showing a marked increase in respiratory activity by a concentration of 1 to 10 parts of ethylene to 10,000 of air, followed by a decrease to or below that of untreated fruit. Denny (*2, 3*) found that concentrations of 1 to 100,000 and 1 to 1,000,000 increased the respiratory activity of lemons. These relatively weak concentrations were used with oranges (fig. 5, *C*) and grapefruit (fig. 7, *C*), but with no significant effect on respiration.

If respiratory activity is an indication of the degreening activity of ethylene, then concentrations of 1 part or less of ethylene to 100,000 parts of air would not be effective for degreening oranges or grapefruit.

On the other hand, the respiratory activity would indicate that 1 part of ethylene in 10,000 of air was as effective as 10 parts per 10,000 and that an initial exposure to ethylene in a confined space was as effective as daily additions of ethylene.

The complete oxidation of a hexose sugar to carbon dioxide and water results in a respiratory ratio of 1.0; a respiratory ratio of 1.33 is obtained from the complete oxidation of citric acid, according to the following equations in which the heat produced is based on gram-molecular weights:

(1)
$$2C_6H_{12}O_6 + 12O_2 \rightarrow 12CO_2 + 12H_2O + 1,348,600 \text{ cal.}$$
Hexose sugar

$$\text{Respiratory ratio} = \frac{CO_2}{O_2} = \frac{12}{12} = 1.0$$

(2)
$$2C_6H_8O_7 + 9O_2 \rightarrow 12CO_2 + 12H_2O + 949,000 \text{ cal.}$$
Citric acid
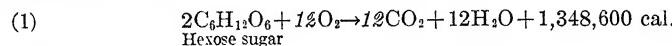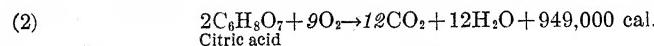
$$\text{Respiratory ratio} = \frac{CO_2}{O_2} = \frac{12}{9} = 1.33$$

Respiratory ratios between 1.0 and 1.33 might represent the respiration of different proportions of sugar and citric acid, according to table 10, assuming that the carbon dioxide evolved and the oxygen consumed are due to the complete oxidation of these materials. However, it has been shown (15) that citrus fruit may evolve ethylene, presumably in the process of respiration. This might be evolved from the oxidation of a hexose sugar, as in the following equation, and would result in a respiratory ratio of 1.33.

(3)
$$2C_6H_{12}O_6 + 6O_2 \rightarrow 8CO_2 + 8H_2O + 2C_2H_4 + 685,400 \text{ cal.}$$
Hexose sugar   Ethylene
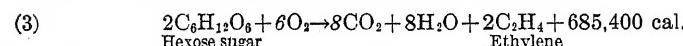
$$\text{Respiratory ratio} = \frac{CO_2}{O_2} = \frac{8}{6} = 1.33$$

TABLE 10.—*Respiratory ratios óbtained by complete oxidation of various proportions of hexose sugars and citric acid*

Hexose sugars	Citric acid	Respiratory ratio	Hexose sugars	Citric acid	Respiratory ratio
Moles	*Moles*		*Moles*	*Moles*	
100	0	1.00	40	60	1.18
90	10	1.025	30	70	1.21
80	20	1.05	20	80	1.25
70	30	1.08	10	90	1.29
60	40	1.11	0	100	1.33
50	50	1.14			

Alcohol has also been found in ripening fruit, particularly in apples (4, 13, 18), and might be formed in the partial oxidation of a hexose sugar or by intramolecular respiration, as in the following equations:

(4)
$$2C_6H_{12}O_6 + 6O_2 \rightarrow 8CO_2 + 6H_2O + 2C_2H_5OH + 693,400 \text{ cal.}$$
Hexose sugar   Alcohol

$$\text{Respiratory ratio} = \frac{CO_2}{O_2} = \frac{8}{6} = 1.33$$

(5)
$$2C_6H_{12}O_6 \rightarrow 4CO_2 + 4C_2H_5OH + 38,200 \text{ cal.}$$
Hexose sugar   Alcohol
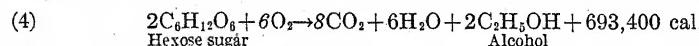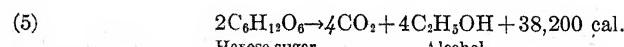
$$\text{Respiratory ratio} = \frac{CO_2}{O_2} = \frac{4}{0} = \infty$$

Thus a respiratory ratio of 1.33 may represent the partial oxidation of a hexose sugar, with the formation of either alcohol or ethylene as one of the end products, as well as the complete oxidation of citric acid.

With respiratory ratios above 1.33, it is probable that a reaction such as that shown in equation 5 enters into the respiration. Thus, a respiratory ratio of 2.0, such as was obtained at 100° F. with Florida oranges (fig. 2, *A*), might represent the intramolecular respiration of 3 moles of a hexose sugar (as in equation 5) and the complete oxidation of 1 mole of a hexose sugar or 2 moles of citric acid (as in equations 1 and 2). Such an equation follows:

(6) $8C_6H_{12}O_6 + 12O_2 \rightarrow 24CO_2 + 12H_2O + 12C_2H_5OH + 1,463,200$ cal.
        Hexose sugar   Alcohol
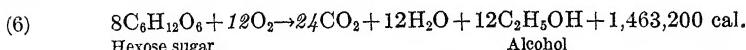
$$\text{Respiratory rate} = \frac{CO_2}{O_2} = \frac{24}{12} = 2.00$$

Acetaldehyde has also been reported (*13, 14, 18*) as an end product in the ripening of apple and lemon fruits and might be formed as in the following equation:

(7) $2C_6H_{12}O_6 + 7O_2 \rightarrow 8CO_2 + 8H_2O + 2C_2H_4O + 790,600$ cal.
        Hexose sugar   Acetaldehyde
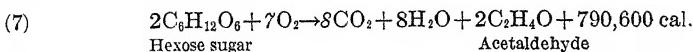
$$\text{Respiratory ratio} = \frac{CO_2}{O_2} = \frac{8}{7} = 1.14$$

This would result in a respiratory ratio equivalent to the complete oxidation of equal molecular weights of a hexose sugar and citric acid (table 10).

From the materials that are known to be present in the fruit and have been shown to occur during the ripening processes, the foregoing reactions might reasonably be expected to represent those occurring during respiration; other reactions doubtless occur also. From these equations it is apparent that increases in the respiratory ratios above 1.0 may be due to other types of respiration than a change from sugar to acid as the material being respired. None of the reactions just indicated results in respiratory ratios above 1.33, except those (equations 5 and 6) in which intramolecular respiration takes place. It seems likely, therefore, that ratios above 1.33 indicate that intramolecular respiration accounts for part of the carbon dioxide evolved.

In intramolecular respiration (equation 5) only one-third as much carbon dioxide is formed as by the complete oxidation of the sugar (equation 1). Thus, a unit of carbon dioxide from intramolecular respiration represents the break-down of three times as much sugar as an equal amount of carbon dioxide produced by the complete oxidation of the sugar, or carbon dioxide evolved at a respiratory ratio of 2.0 (equation 6) might represent the break-down of twice as much sugar as at a ratio of 1.0.

The numbers of calories given in equations 1 and 2, for the complete oxidation of hexose sugars and citric acid, were taken from Hodgman (*8*). The heat of combustion for fructose is slightly greater (0.38 percent) than that for dextrose, and the average for the two is given. The numbers of calories for the other equations, in which intermediate products were formed, were obtained by deducting the heat of combustion of the intermediate product from

the heat of combustion of complete oxidation. In equation 3, for example, the heat of combustion of 2 gram molecules of ethylene is 663,200 calories. This deducted from the heat of combustion of 2 gram molecules of sugar $(1,348,600-663,200)$ gives 685,400 calories as the heat of combustion for equation 3, in which ethylene is formed.

The heat of respiration has generally been computed from carbon dioxide measurements, on the assumption that the carbon dioxide was evolved from the complete oxidation of the hexose sugar dextrose (*16*), as in equation 1. Haller et al. (6) have pointed out that this introduces an error of about 30 percent if the carbon dioxide comes from the complete oxidation of citric or malic acid. Since considerable acid is generally lost during ripening after harvest, it is likely that much of the carbon dioxide comes from the oxidation of the acid, with a proportionate error in the computation of the heat of respiration. Similarly, if the carbon dioxide comes from the partial oxidation of the sugar, as in equations 3, 4, and 7, the actual heat of respiration would be much lower (12 to 24 percent) than the calculated heat of respiration (table 11). With partial intramolecular respiration, as in equation 6, the error would be very large (46 percent).

TABLE 11.—*Heat of respiration per unit of CO_2 evolved and O_2 consumed from various probable respiratory reactions*

Equation No.	Heat of respiration per unit of—		Equation No.	Heat of respiration per unit of—	
	CO_2	O_2		CO_2	O_2
	Calories	*Calories*		*Calories*	*Calories*
1	112,383	112,383	5	9,550	
2	79,083	105,444	6	60,907	121,933
3	85,675	114,233	7	98,825	112,943
4	86,675	115,567			

On the other hand, the heat evolved per unit of oxygen consumed varied relatively little (table 11), regardless of the type of reaction involved. If the complete oxidation of a hexose sugar (equation 1) is again used as a standard for calculating the heat of respiration, the errors due to the type of reaction would be relatively slight when computed from the oxygen measurements. The lowest heat of respiration or combustion per unit of oxygen results from the complete oxidation of citric acid (table 11, equation 2), and this was only 6.4 percent lower than the standard when calculated from the oxygen determinations, as contrasted with a 30-percent reduction when calculated from the carbon dioxide determinations. The highest heat of respiration per unit of oxygen was obtained with intramolecular respiration, and with a respiratory ratio of 2.0 (as in equation 6) the actual heat of respiration would be only 8.5 percent higher than the standard if computed from oxygen determinations, as contrasted with results 46.0 percent lower than the standard if computed from carbon dioxide determinations.

From these considerations it is apparent that the heat of respiration as generally computed from carbon dioxide determinations may be highly erroneous unless the reaction involved in its production is known and the computation is based on the specific reaction in-

volved. On the other hand, the heat of respiration per unit of oxygen consumed is fairly constant, regardless of the type of reaction concerned. This emphasizes the importance of determining the oxygen consumed, as well as the carbon dioxide evolved, in respiration determinations in which the calculation of the heat of respiration is of interest.

However, in connection with these calculations, attention should be given to the results obtained by Green et al. (*5, pp. 20–21*) during an investigation of a number of fruits and vegetables held at 45° and 65° F., in which they calculated the heat of respiration from the carbon dioxide evolved and determined the heat by calorimetric measurements. They concluded that ". . . the values for the heat produced which are obtained by calculations based on the rate of production of carbon dioxide are within 10 percent of those obtained by means of the calorimeter herein described." With two lots of sound oranges they found the calculated values to be only 3 and 5 percent higher than those obtained by means of the calorimeter. Their results do not show divergencies between calculated and measured heat of respiration as great as those indicated from theoretical consideration of possible reactions (table 11). It is likely that respiration in citrus fruits usually consists of not one but several of the reactions indicated, with the complete oxidation of a hexose sugar (equation 1) predominating, so that the heat of respiration calculated from the carbon dioxide determinations would not differ greatly from the actual heat evolved. The error due to such calculations is not likely to be great, except at high temperatures at which intramolecular respiration occurs.

SUMMARY

The carbon dioxide evolved and the oxygen consumed by oranges, grapefruit, and lemons were determined under different experimental conditions.

The respiratory rates of all the fruits increased greatly with increased temperature. The temperature coefficients (Q_{10}) were highest between 32° and 50° F.; dropped to minima, averaging about 1.5 between 62° and 90°; and increased again above these temperatures, particularly in carbon dioxide output.

At the lower temperatures (32°, 40°, and 50° F.), the respiratory rates remained fairly constant with time, whereas at the higher temperatures (70° to 110°) the rates generally decreased with time. The respiratory ratios $\frac{\text{volume of } CO_2}{\text{volume of } O_2}$ did not differ significantly at the low and intermediate temperatures (32° to 80°) but increased markedly at the higher temperatures (90° to 110°), indicating intramolecular respiration.

The results indicate that the respiratory activity of oranges and lemons decreases with increased maturity.

The respiratory activity of oranges from trees on sour orange rootstocks was higher than that of oranges from trees on rough lemon rootstocks.

The heat of respiration of the different fruits was computed for the different temperatures. The heat of respiration averaged 11 to 96 percent higher when computed from carbon dioxide determinations

than when computed from oxygen determinations. It is pointed out that oxygen determinations represent the sounder basis for computing the heat of respiration, particularly at high temperatures.

The addition of ethylene, at the rates of 1 to 10 parts by volume to 10,000 parts of air, greatly increased the respiratory activity of oranges, grapefruit, and lemons. The increase was as much with 1 as with 10 parts of ethylene per 10,000; much weaker concentrations (1 part to 100,000 and 1 part to 1,000,000) had no apparent effect. Daily additions of ethylene at the rate of 2 parts per 10,000 did not increase or maintain the respiratory activity more than the initial addition of 2 parts per 10,000. The maximum respiratory activity was generally attained 2 to 3 days after the addition of ethylene; the activity then decreased; and respiration returned to normal after aeration.

The respiratory activity of lemons infected with green mold (*Penicillium digitatum*) was increased to as much as 12 times that of sound lemons.

The results indicated that respiratory activity occurs in both the flesh and rind of lemons approximately in proportion to their fresh weights.

The respiratory activity of grapefruit from trees sprayed with lead arsenate was lower than that of grapefruit from trees not so sprayed.

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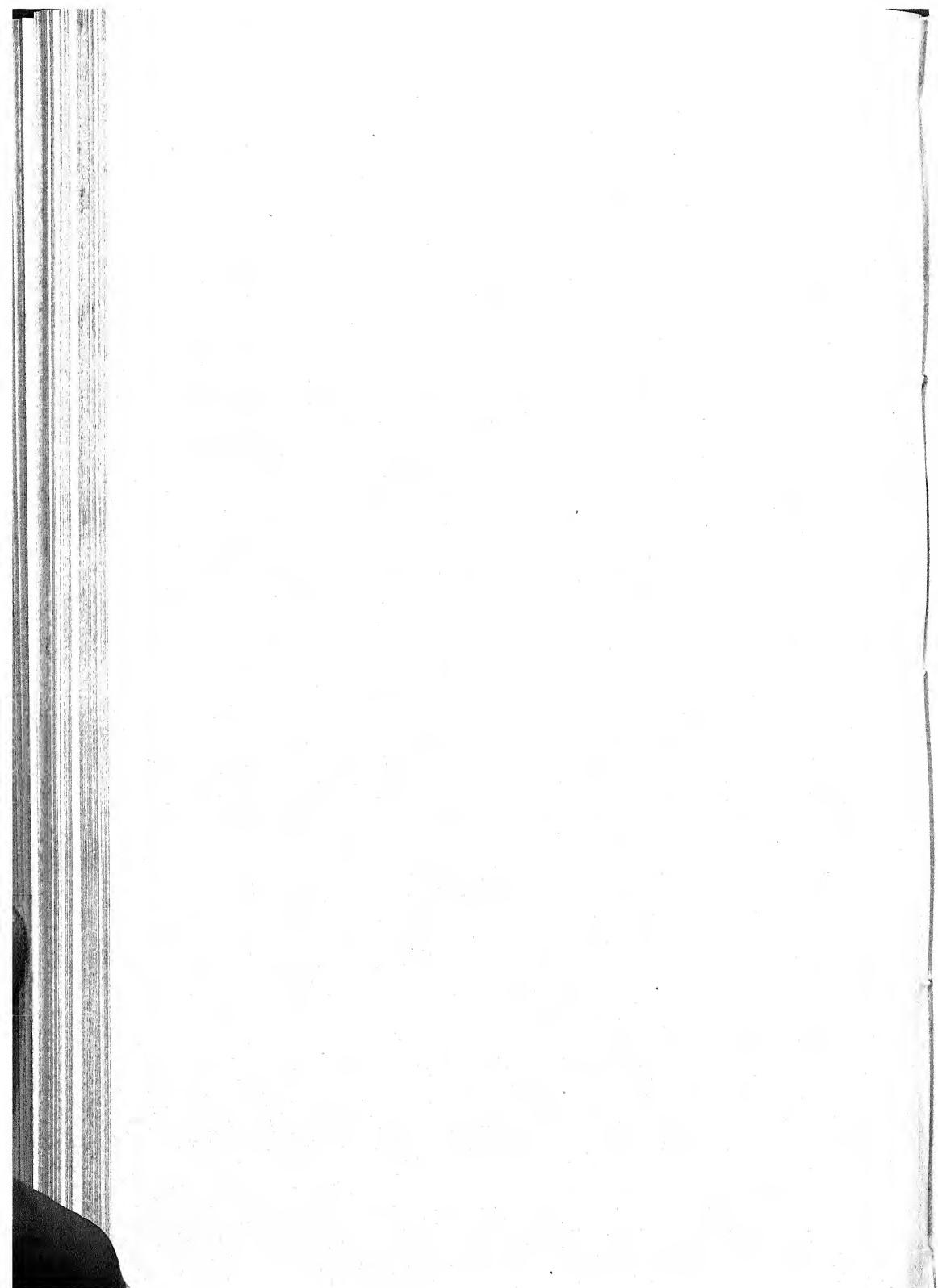
RETENTION OF CAROTENE IN ALFALFA STORED IN ATMOSPHERES OF LOW OXYGEN CONTENT [1]

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INTRODUCTION

Partly because of its relatively high carotene content, alfalfa has long been recognized as of exceptional value in animal nutrition. Alfalfa meal as a source of carotene, or provitamin A, has increased in importance with increased demands for this vitamin and the threatened shortage of such concentrates as fishliver oils. Obviously preservation of the carotene content of alfalfa is important both in securing the greatest benefits from its use as a feedstuff and in economical recovery of the carotene.

Artificial drying or dehydration has been found effective in minimizing the losses of carotene that ordinarily accompany the curing of alfalfa in the field, but both the field-cured and the dehydrated product lose carotene rapidly under present-day methods of storage in air.

It is generally recognized that the loss of carotene in alfalfa is an oxidation process,[2] and numerous investigations have been made of the relations of time and temperature to the extent of carotene loss in air. But very little consideration has been given to the obviously important factor of oxygen exclusion in alfalfa storage. Taylor and Russell[3] found that storage of alfalfa meal in vacuo at approximately 0° C. was very effective in reducing carotene loss. Samples of alfalfa ground in a Wiley mill to pass a 1-mm. screen and containing 0.014 percent of carotene, when stored for 12 to 20 months in vacuo at $0 \pm 5°$ C. had a high degree of carotene stability. There was a loss of 23 percent in 12 months. Samples more finely ground in a ball mill, under the same conditions, suffered very little change in caretone throughout a 20-month period. On the other hand, a sample (Wiley-milled) kept in the dark at $0 \pm 5°$, in a sealed bottle containing air, lost 70 percent. Moreover, samples kept in the dark at room temperature, in air in sealed bottles, lost 87 percent (Wiley-milled) and 77 percent (ball-milled). It was thus shown that vacuum storage was very effective in preserving the carotene content of alfalfa.

Vacuum storage did not appear to be economically feasible, but storage in a gas that is free from, or low in, oxygen seemed likely to produce the same result as vacuum storage, at less expense. Controlled gas atmospheres are used commercially in the storage of fruit.

A search of the literature on gas storage and on refrigerated gas storage revealed that much has been published with respect to such

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storage of fruits, vegetables, and meats; but no method was found in use or suggested for the commercial storage of alfalfa in an inert atmosphere or one of limited oxygen content. Accordingly the present investigation was undertaken to establish a basis for pilot-plant studies.

During the progress of this investigation the work of Wilder and Bethke[4] was published. These investigators found that the carotene in machine-dried alfalfa meal was stable when stored in sealed cans in vacuo or in an atmosphere of nitrogen at room temperature and at 37° C. Little or no loss of carotene occurred.

The investigation of Taylor and Russell had shown that cold storage reduced carotene loss in air in sealed containers from 87 percent at room temperature to 70 percent at approximately 0° C. It had further shown the effectiveness of vacuum storage in conserving carotene. The work of Wilder and Bethke confirmed the results of the former investigators with respect to storage in vacuo, and further indicated the effectiveness of storage in nitrogen at room temperature and at 37° C. In the report of their investigations, however, Wilder and Bethke do not state the extent of oxygen exclusion by evacuation or by replacement of air with nitrogen.

PLAN OF THE INVESTIGATION

Since the purpose of the present investigation was to provide a basis for commercial application, it was essential to acquire quantitative information on the degree of oxygen exclusion required for preservation of carotene. For this reason the investigation as originally planned had for its object a comparison of carotene losses in samples of alfalfa meal stored in closed containers containing atmospheres of different oxygen content, ranging from air to nitrogen containing 0.3 percent of oxygen, under as nearly identical conditions of temperature and relative humidity as possible. A temperature of 40° C. and a relative humidity of 40 percent were selected as typical of those generally prevailing in summer in the valleys of California and some other alfalfa-producing areas. Later the investigation was expanded to include the effect of carbon dioxide in the storage atmosphere, because this gas might be used as the so-called inert gas or as a constituent of other commercial inert gases. Also determination of the effect of a daily renewal of the storage atmosphere was included.

The three phases of experimentation carried out were as follows:

(1) Losses of carotene in alfalfa meal in closed containers in air [5] and in atmospheres consisting of nitrogen and 5.3 percent oxygen, 3.0 percent oxygen, 1.6 percent oxygen, and 0.3 percent oxygen, at 40° C. and a relative humidity of approximately 40 percent.

(2) The effect of 13 percent of carbon dioxide in the 3-percent-oxygen atmosphere. (The remainder was nitrogen.)

(3) The effect of a daily renewal of the 3-percent-oxygen atmosphere containing the 13 percent of carbon dioxide. This experiment may be considered as simulating conditions in commercial storage in an inert gas; i. e., considerable leakage of the gas and entrance of outside air.

[4] WILDER, O. H. M., and BETHKE, R. M. THE LOSS OF CAROTENE IN MACHINE-DRIED ALFALFA MEAL UNDER VARIABLE CONDITIONS OF STORAGE. Poultry Sci. 20: 304–312, illus. 1941.

[5] A preliminary comparison of carotene losses in air at 40° and 25° C. under these conditions had been made. At 40° the samples of a low-grade alfalfa meal lost 45 percent of the carotene in 67 days; at 25° samples of the same meal lost 30 percent in 69 days.

EXPERIMENTAL PROCEDURE

ALFALFA MEAL, PRELIMINARY TREATMENT, AND SAMPLING

The alfalfa meal [6] used in the experiments was prepared from dehydrated alfalfa on August 1, 1941. The alfalfa (fourth cutting) was about 30 days old when cut. Practically all the meal, on screening test, passed through an 18-mesh screen, 65 percent through a 60-mesh, 34 percent through a 120-mesh, and 19 percent through a 200-mesh. As received, on August 4, the meal contained 8.3 percent of moisture and had a carotene content of approximately 225 parts per million.

In order to preserve the carotene content and to promote the establishment of moisture equilibrium at the relative humidity selected for the experimental storage atmospheres, a sufficient quantity of the meal for all anticipated requirements was stored at 0° C., in uncovered dishes placed in large desiccators over sulfuric acid of 46.9-percent strength. From this common source, the meal was removed as required for the tests.

The containers used for the samples in storage were glass U tubes, Schwartz type. These were tested against leakage under moderate pressure. The volumetric capacity of the eight tubes used for storage in nitrogen varied from 26.2 to 28.1 cc. For storage in the other atmospheres the volumes ranged from a minimum of 19.8 to a maximum of 25.1 cc. The weights of samples placed in the tubes were all in the same proportion of meal to volumetric capacity, i. e., almost exactly 1 gm. of meal per 4.78 cc. of volume. A rough estimate showed that the loose meal occupied nearly 60 percent of the enclosed space.

A uniform procedure was followed in introducing the required quantities of samples into the U-tubes. The tubes were weighed, filled, and reweighed before the stopcocks were greased. The calculated quantity of meal required for a given tube was first weighed out in a covered weighing bottle to within 0.1 gm. This was then transferred carefully through a paper funnel into the previously weighed tube. Reweighing of the tube gave the exact weight of sample transferred. The stopcocks were then greased lightly with vaseline and the tube was reweighed. This last weight was used in determining any subsequent changes in weight before and at the conclusion of storage.

For each series of experiments, in air, nitrogen, etc., 12 samples of meal in the same number of U tubes were prepared in the manner described. All of these samples were subjected to the same treatment up to the beginning of storage. Four of the samples were then separated, 2 being used for determinations of moisture and 2 for carotene determinations at the beginning of storage. The remaining 8 samples were used for the various periods of storage.

THE APPARATUS

The apparatus used in the preparation of the gas mixtures and for the conditioning of the alfalfa samples for storage is shown in figures 1 and 2. In figure 1, *A* and *B* are 5-gallon bottles filled with water and connected as shown. *D* is a constant temperature water bath with heater, stirrer, bimetallic regulator, contrivance for adjusting inflow and outflow of running water (at a temperature below 23° C.),

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[6] Obtained through the courtesy of Dr. George Kernohan, of Poultry Producers of Central California.

and the humidifier E. This humidifier is a specially constructed gas-washing bottle of spiral type filled with water. O is an electric oven in which the U tubes are placed, connected in series and attached to the apparatus at h and k. W is a wash bottle containing approximately 2 inches of water for saturating the emerging gas before it passes through the wet test meter (M). The pressure in the system is indicated by a small manometer (p).

When the gas desired for the storage atmosphere was air or nitrogen, this was introduced directly into the bottle A through stopcock d by way of e by displacement of the water into C, a large aspirator bottle. The other gas mixtures required contained a lower proportion of oxygen than that in air. They were prepared by introducing into A from an additional measuring bottle equal volumes of air and
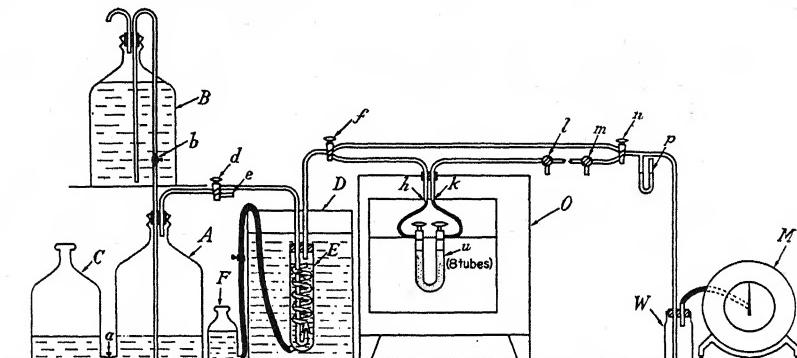
FIGURE 1.—Apparatus for gas aspiration and humidity control.

nitrogen, thus producing a gas containing approximately 10 percent of oxygen. Then successive dilutions of this mixture with nitrogen were made by the same procedure. For the preparation of the atmosphere of carbon dioxide, oxygen, and nitrogen it was found simpler to introduce nitrogen into bottle A to a previously determined level of water corresponding to a known volume of gas and to follow this by the introduction of the volumes of oxygen and carbon dioxide required for the concentrations desired. In operation, the gas in bottle A was displaced by water from bottle B through stopcock b.

PREPARING THE SAMPLES FOR STORAGE IN THE DIFFERENT ATMOSPHERES

A relative humidity of approximately 40 percent at 40° C. was desired for the storage atmospheres. The preliminary storage of the meal at 0° C. in desiccators over sulfuric acid of the proper strength tended to contribute to this end. After it was sampled and weighed into the U tubes, the meal, surrounded by air, was subjected to two operations. First, the 12 tubes were connected in series and 7 liters of air, conditioned by passage through gas-washing bottles containing sulfuric acid of 48.4 percent strength, was passed through the tubes for a period of approximately 6 hours. Previous experiments had indicated that by this operation at room temperature (approximately 25° C.) practically constant weights of the samples were attained. However, an average relative humidity of 46.5 percent instead of 40 percent for the atmospheres at the end of two such operations was found experimentally. This higher relative humidity

can be explained by the observed fact that in the determinations of relative humidity there was a small net loss in the moisture content of the alfalfa samples.

The apparatus used in this operation is shown in figure 2. *A* is a long glass tube containing sulfuric acid of approximately 48 percent strength set up for control of air pressure in *B* and *C*, two gas-washing bottles containing sulfuric acid of 48.4 percent strength. *T* is a U tube filled with glass wool to trap any spray of acid from *C*, and *U* represents the series of 12 U tubes. At the completion of this operation all the tubes were again weighed. Two of the samples were used for the determination of moisture and 2 for the carotene analysis. The moisture and carotene content of these samples was assumed to be applicable to each of the remaining 8 samples.
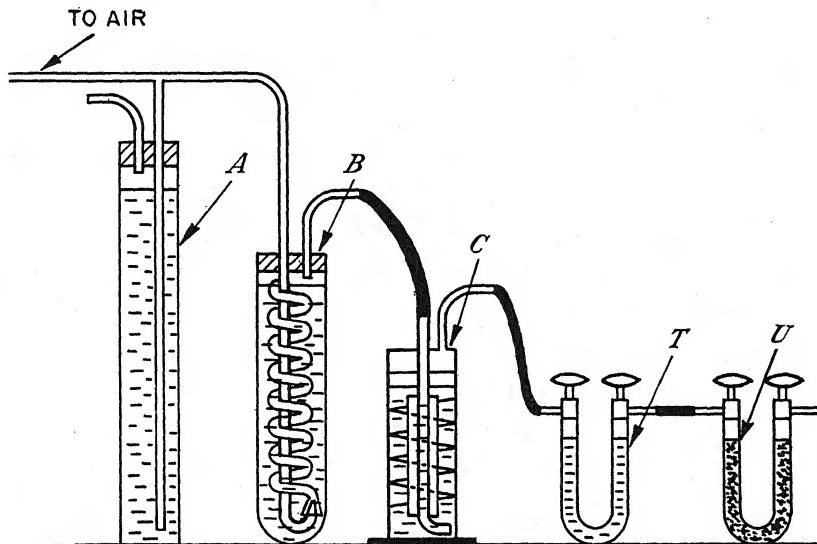
FIGURE 2.—Apparatus for preconditioning alfalfa to 40 percent relative humidity at room temperature.

The second operation consisted in placing the eight samples in the oven at 40° C. and establishing the desired atmosphere in the tubes. Previous tests had shown that air could be displaced in the eight tubes by the passage of 2.6 liters of nitrogen in 2 hours. As an added precaution, 4 liters of the desired atmosphere was passed in 3 to 4 hours.

The procedure followed for the production of a relative humidity of approximately 40 percent at 40° C. was based on the calculation that a gas saturated with water vapor at 23.8° C. and then heated to 40° should have a relative humidity of 40 percent at the higher temperature. This calculation did not take into account any change in the moisture equilibrium of the alfalfa at the higher temperature. A determination of the relative humidity produced by this procedure was made and it was found to be 45 percent. Here, again, as at room temperature, a small loss in total moisture of the samples was noted at the completion of the determination.

It would have proved detrimental to the accuracy of the determinations of carotene loss in storage to have made determinations of

relative humidity of the atmospheres in which the samples were actually stored, and consequently it can only be assumed that under identical conditions of procedure the same relative humidity prevailed in the atmospheres of all samples.

Before passage of gas through the samples was begun in O (fig. 1), the humidifier (E) was filled with water to displace air by raising the leveling bottle (F). The displaced air was by-passed through W and M by the use of the three-way stopcocks, f and n. The gas was then drawn in from A by lowering F and displaced in the same manner several times to flush out air in the apparatus between stopcocks d and f. The water in E was then lowered approximately 1½ inches from the rubber stopper, thus drawing in fresh gas, and the screw cock between E and F was closed. The desired rate of gas flow was established by by-passing it from A through stopcocks d, f, and n.

The temperature of the bath (D) was adjusted to 23.8° C. The sample tubes were placed in the oven, connected in series, and attached to the apparatus at h and k. The rate of gas flow was kept fairly uniform by control of the water level in B and adjustment of stopcock d. After the passage of approximately 4 liters of gas in 3 to 4 hours, the flow of gas was discontinued. For a few seconds the gas was allowed to escape at l through a drying tube to bring the pressure of the storage atmosphere to atmospheric. The stopcocks of all the tubes in the oven were then quickly closed.

The last-described procedure was slightly modified for the gas mixture containing 13 percent carbon dioxide, 3 percent oxygen, and 84 percent nitrogen. In this case, instead of water in bottles A and B and the humidifier, a 20-percent solution of sodium chloride saturated with the gas mixture was used, and the temperature of the water bath was maintained at 26.6° C. to take care of vapor-pressure relations.

In the experiment designed to show the effect of daily renewal of the storage atmosphere, the same concentrations of carbon dioxide and oxygen were used as in the preceding experiment with carbon dioxide in the closed containers. The procedure was also the same except that daily except Sundays a volume of the gas equal to twice the capacity of the tubes in the oven was passed through in the manner described.

At selected intervals, from 14 to 112 days, comparable for the respective atmospheres, samples were removed from the oven, weighed for comparison with original weights, and analyzed for carotene.

THE DETERMINATION OF CAROTENE

Carotene was determined by a slight modification of the method described by Kernohan.[7] Because of its simplicity and the comparatively brief time required, the method was thoroughly investigated and found to meet fully the requirements for consistent and precise determinations of small changes in the carotene content of the alfalfa.

The method specifies overnight digestion of the sample with petroleum ether. A longer digestion (48 hours) was found to yield slightly higher values for carotene (1 to 7 parts per million higher). In the first experiments the filtering column of soda ash used was 3½ inches

[7] KERNOHAN, G. THE DETERMINATION OF CAROTENE. Science 90 : 623. 1939.

long, but the results were unsatisfactory because not all of the non-carotene coloring matter was removed. The use of a 6-inch column eliminated this inaccuracy, and the increase in amount of absorbent caused no retention of carotene by the soda ash when a Skellysolve solution of pure carotene was filtered.

The procedure adopted is as follows: Digest the alfalfa sample (1.0–1.3 gm.) at room temperature for 48 hours with 100 cc. of Skellysolve. Filter the solution through a 6-inch column of soda ash. Wash the filter first with 50 to 60 cc. of the solvent and then with
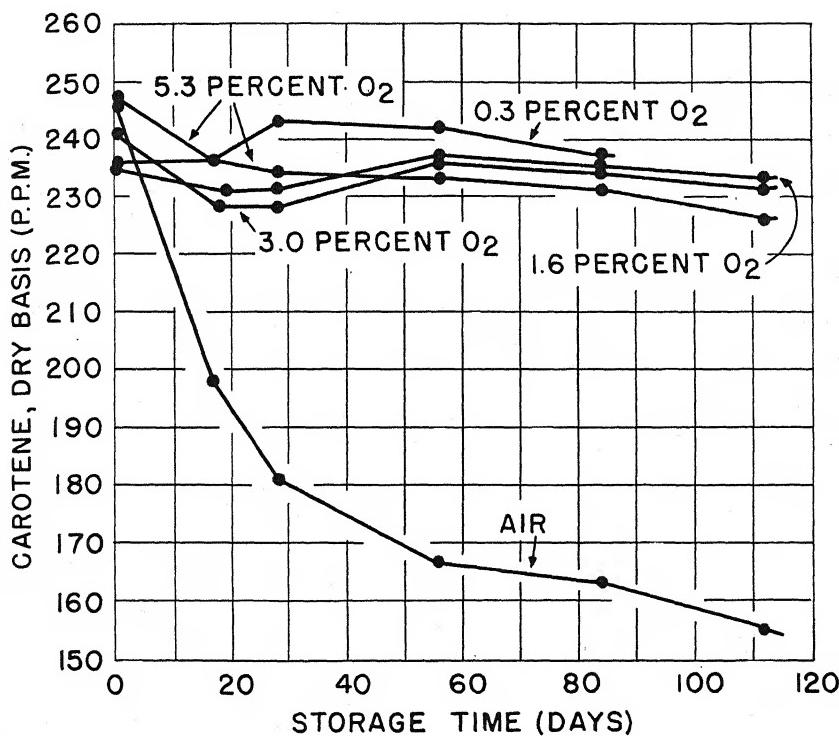
FIGURE 3.—Losses in carotene content of alfalfa meal stored in nitrogen containing various amounts of oxygen. (For the computed probable errors at individual points on the curves see table 1.)

35- to 40-cc. portions until the 250-cc. volumetric flask is filled to the mark, using slight air pressure upon the top of the column. Determine carotene by the use of a photoelectric colorimeter calibrated against solutions of pure carotene. In the writers' determinations, an Evelyn photoelectric colorimeter with a 420-mμ filter was used. The 6-inch filtering column had a diameter of 47 mm. The weight of soda ash (grade 58 percent, light) required for a column of these dimensions varied somewhat with the fineness of the soda ash (120 to 150 gm.).

RESULTS

The results of the storage tests are shown in tables 1 and 2 and graphically in figures 3 and 4. These tests were begun in successive

weeks. They were conducted first in air, and then in mixtures of nitrogen and oxygen with progressively decreasing proportions of oxygen (table 1); 5 months after the beginning, the tests with carbon dioxide were started (table 2). This fact accounts for the variation in original carotene values in the different series. The moisture content of the alfalfa samples ranged from 8.1 to 8.6 percent.
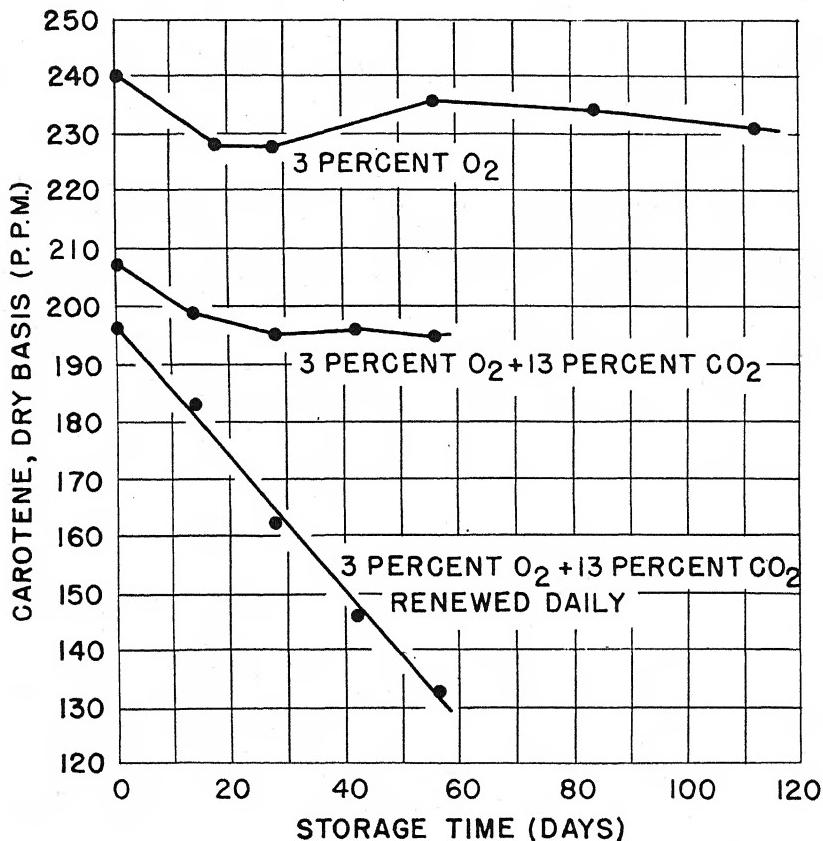
FIGURE 4.—Losses in carotene content of alfalfa meal stored in nitrogen containing various amounts of carbon dioxide and oxygen, with and without daily renewal of atmosphere. (For the computed probable errors at individual points on the curves see table 2.)

The data given in table 1 and figure 3 show definitely that a reduction in the oxygen content of the storage atmosphere tends to preserve the carotene in alfalfa. In air there was a progressive loss of carotene from 19.8 percent in 17 days to 37.3 percent in 112 days. In an atmosphere containing 5.3 percent of oxygen, the loss of 4.8 percent in 17 days increased to 8.9 percent in 112 days. With 3 percent oxygen, the loss of carotene reached its maximum of 5.8 percent in 18 days. The same loss was recorded at 28 days. The loss at 56 days had decreased to 2.5 percent, after which the loss was progressive up to 4.6 percent in 112 days.

TABLE 1.—*Carotene content of alfalfa meal and losses during storage in atmospheres of different oxygen content*

Length of storage (days)	Carotene content for storage atmosphere of—														
	Air			Oxygen, 5.3 percent; nitrogen, 94.7 percent			Oxygen, 3 percent; nitrogen, 97 percent			Oxygen, 1.6 percent; nitrogen, 98.4 percent			Oxygen, 0.3 percent; nitrogen, 99.7 percent		
	Dry basis [1]	Probable error [2]	Loss	Dry basis [1]	Probable error [2]	Loss	Dry basis [1]	Probable error [2]	Loss	Dry basis [1]	Probable error [2]	Loss	Dry basis [1]	Probable error [2]	Loss
	P.p.m.	*P.p.m.*	*Pct.*	*P.p.m.*	*P.p.m.*	*Pct.*	*P.p.m.*	*P.p.m.*	*Pct.*	*P.p.m.*	*P.p.m.*	*Pct.*	*P.p.m.*	*P.p.m.*	*Pct.*
0	[3] 252 / [3] 242	6	0	[3] 248 / [3] 248	6	0	[3] 238 / [3] 246	5	0	234 / 236	3	0	236 / 235	3	0
17	198	7	19.8	[4] 236	1	4.8	[4] 228	4	5.8	[4] 231	3	[6] 1.7	[4] 236	4	0
28	181	3	26.7	236	5	5.7	230	3	5.8	234	3	1.7	244	4	+3.0
56	166 / 166	3	32.8	231 / 234	5	6.1	227	4	2.5	229 / 238	3	+.9	241 / 241	3	+2.5
84	167 / 159	4	34.0	232 / 229	4	6.9	[3] 234 / 238 / 232	6	3.3	236 / 236	3	0	243 / 236	8	+.4
112	155	4	37.3	[4] 226	4	8.9	237 / [4] 231	5	4.6	234 / [4] 233	9	.9	238		

[1] Average of 3 determinations on contents of same sample tube, except where indicated.

[2] Probable error in determination of carotene computed from Peters' formula: $R = \frac{0.845}{\sqrt{n(n-1)}} \Sigma x$, where n = number of observations and x = individual deviation from arithmetical mean.

[3] Average of 2 determinations on contents of same sample tube.

[4] Average of 4 determinations on contents of same sample tube.

[5] 18 days' storage.

[6] 19 days' storage.

TABLE 2.—*Carotene content of alfalfa meal and losses during storage in atmospheres consisting of various amounts of nitrogen, carbon dioxide, and oxygen, with and without daily renewal of atmosphere*

Length of storage (days)	Carotene content for storage atmospheres of—											
	(From table 1) Air			(From table 1) Oxygen, 3 percent; nitrogen, 97 percent			Oxygen, 3 percent; carbon dioxide, 13 percent; nitrogen, 84 percent			Oxygen, 3 percent; carbon dioxide, 13 percent; nitrogen, 84 percent—renewed daily		
	Dry basis	Probable error [1]	Loss	Dry basis	Probable error [1]	Loss	Dry basis [2]	Probable error [1]	Loss	Dry basis [2]	Probable error [1]	Loss
	P.p.m.	P.p.m.	Pct.	P.p.m.	P.p.m.	Pct.	P.p.m.	P.p.m.	Pct.	P.p.m.	P.p.m.	Pct.
0	247	6	0	242	5	0	211, 204	4	0	197, 197	2	0
14							201, 197	3	4.3	185, 181	2	7.1
28	181	3	26.7	228	3	5.8	194, 195	1	6.3	162, 161	2	17.8
							198, 193	3	5.8	147, 145	2	25.9
56	166	3	32.8	236	4	2.5	194, 195	3	6.3	133, 132	3	32.5

[1] Probable error in determination of carotene computed from Peters' formula: $R = \dfrac{0.845}{\sqrt{n(n-1)}}\Sigma z$, where n = number of observations and z = individual deviation from arithmetical mean.

[2] Average of 3 determinations on contents of same sample tube.

In an atmosphere of 1.6 percent oxygen a maximum loss of 1.7 percent occurred in 19 and 28 days. After 28 days, the results were parallel to those of the 3.0 percent-oxygen atmosphere, where there was an apparent increase in carotene, followed by a decrease to a final loss of one-half that of 19 days. In nitrogen containing 0.3 percent oxygen no loss whatever was found. On the other hand, there was an apparent increase in carotene content between 17 and 28 days of 3.0 percent, which increase was reduced to 0.4 percent at the end of 84 days.

The deviations from progressive carotene loss are shown in figure 3. All the curves follow the same general pattern. For storage in nitrogen (containing 0.3 percent oxygen), the curve begins its upward trend at 17 days; from 28 days the trend is definitely downward. For 1.6
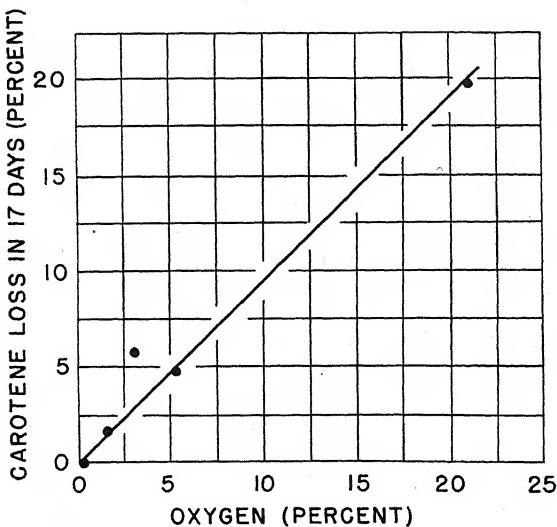
Figure 5.—Relation of initial carotene loss to oxygen content of storage atmosphere.

percent oxygen the trend upward begins at 28 days and downward at 56 days. The same is true for 3.0 percent oxygen. For 5.3 percent oxygen, although there is no definite upward trend at any time, from 28 days to 112 days a reduced rate of carotene loss is indicated. Even for air, after 56 days, the curve follows somewhat the same pattern as for 5.3 percent oxygen.

Table 2 and figure 4 show the effect of the presence of carbon dioxide in an atmosphere of 3.0 percent oxygen, and also the effect of daily renewal of this atmosphere. Comparison of these results with those of the 3.0-percent-oxygen atmosphere without carbon dioxide (table 1) shows (1) that the presence of carbon dioxide in this atmosphere is of no significance in the preservation, or loss, of carotene; (2) that a daily renewal of the storage atmosphere results in a progressive and decidedly greater loss of carotene (32.5 percent), almost exactly the loss occurring in the same length of time (56 days) in air.

When the percentages of carotene losses in 17 days are plotted against the percentages of oxygen in the storage atmosphere (fig. 5), it is seen that, with one exception (the 3.0 percent oxygen) the points

lie on, or are very close to, a straight line passing through the origin. It may therefore be concluded that at least the initial rates of carotene destruction are very nearly proportional to the percentages of oxygen in the storage gases.

The apparent gains in carotene content in nitrogen (containing 0.3 percent oxygen) after 17 days, and the similar trend in atmospheres containing 1.6 and 3.0 percent oxygen, cannot be explained as due solely to the limits of experimental accuracy of the determinations. In all the analyses in which the carotene content of the sample in each of two tubes stored for the same length of time in the same atmosphere was determined in triplicate, the average difference in carotene between the two tubes was less than 4 parts per million, the maximum being 5 parts per million (in 2 out of 9 cases). The average of the maximum differences in individual determinations of carotene made in triplicate for each tube was 11 parts per million. This difference can be accounted for by variations in the triplicate samples taken from the contents of the tube, but error from this cause would be expected to disappear largely in the average of the three determinations representing 60 to 80 percent of the total contents of the tube.

A similar apparent increase in the carotene content was reported by Wilder and Bethke [8] for samples of alfalfa meal in nitrogen-packed, metal containers stored at room temperature (between 21° and 23° C.) and at 37° C. and likewise for samples packed in vacuo, in similar containers, and stored at the same temperatures.

The writers do not believe that there was an actual increase in carotene in atmospheres of low oxygen content. On the other hand, an application of calculated probable error of the analytical determinations of carotene to each point of the curves (fig. 3) is not sufficient to eliminate the general upward trend in the curves. The cause of the apparent increase in carotene during storage in atmospheres of low oxygen content cannot be explained. The formation of coloring material that behaves like carotene in the physical method used for carotene estimation can be assumed to be the cause; however, such formation of color might also take place in atmospheres of higher oxygen content but be overlooked because of the destruction of more color by oxidation of carotene. This would explain why the upward trend in the curves appears earlier in cases of storage in low concentrations of oxygen. Whatever may be the explanation, the results of the carotene determinations for alfalfa stored in atmospheres of different oxygen content would still remain relatively comparable with each other, and would unquestionably indicate the least loss of carotene from storage in the atmosphere of lowest oxygen content.

Carotene losses were greatly reduced in the atmospheres of low oxygen content in the closed containers. The percentage loss of carotene resulting from the daily renewal of a 3-percent-oxygen atmosphere for 56 days was as great as that in air in the closed containers for the same number of days. From these facts the conclusion was reached that storage in the closed containers was accompanied by a reduction in oxygen concentration in the initial stages of storage through oxidation of constituents of the alfalfa other than carotene, and in consequence loss of carotene was retarded or prevented entirely.

[8] See footnote 4, p. 362.

The correctness of this conclusion was confirmed by the following experiment:

A mixture of air and nitrogen containing approximately 3 percent of oxygen was prepared. Two large U tubes of 410-cc. capacity provided with vacuum-tight stopcocks were used as containers for the alfalfa; 85.8 gm. from the same lot of alfalfa as that previously used was weighed into each of the U tubes, this weight being that required for the same proportion of meal to capacity of container as in the previous experiments. In addition to these samples four other samples in small U tubes were provided for carotene and moisture determinations. The tubes were conditioned for storage by the passage of 8.7 liters of the gas in 6⅓ hours at room temperature, and following this operation the large tubes were placed in the oven at 40° C. and conditioned for humidity by the passage of 6 liters of the gas in 5⁵⁄₁₂ hours, the gas first having passed through a 20-percent solution of sodium chloride at 26.7° in the humidifier shown in figure 1. The gas emerging from the tubes at the end of this operation was found to contain 3.25 percent of oxygen, and this value for oxygen was recorded as that of the storage atmosphere. The tubes were then closed and left in the oven for 28 days. At the end of 28 days they were removed from storage, and samples of the gas were collected under reduced pressure from the tubes and analyzed. Carotene and moisture in the alfalfa after storage were also determined.

In both tubes the oxygen had been completely consumed. The carotene loss was only 11 parts per million, or 5.6 percent, which is far too small to account for the oxygen consumption. This loss is in remarkably close agreement with the losses in 28 days in approximately the same concentration of oxygen recorded in tables 1 and 2. The carbon dioxide produced was found to be in excess of the oxygen consumption, being 5.8 and 5.7 percent in the two tubes.

SUMMARY AND CONCLUSIONS

The storage of alfalfa meal at 40° C. in closed containers in contact with atmospheres of low oxygen content resulted in a high degree of carotene conservation. The presence of carbon dioxide in these atmospheres had little effect.

Conservation of carotene was due in large measure to a reduction in the original oxygen concentration by oxidation of constituents of the alfalfa other than carotene in the early stages of storage.

A daily renewal of the storage atmosphere containing oxygen was accompanied by a great increase in carotene loss.

It therefore follows that the most effective conservation of carotene in alfalfa commercially by means of inert gas would be accomplished by the highest possible exclusion of air throughout the entire storage period. The extent to which oxygen can be excluded from the storage atmosphere economically in commercial practice remains to be investigated. It would appear from a comparison of the curves in figure 4 that the permissible oxygen content of the storage atmosphere at the beginning will be somewhat less than 3 percent.
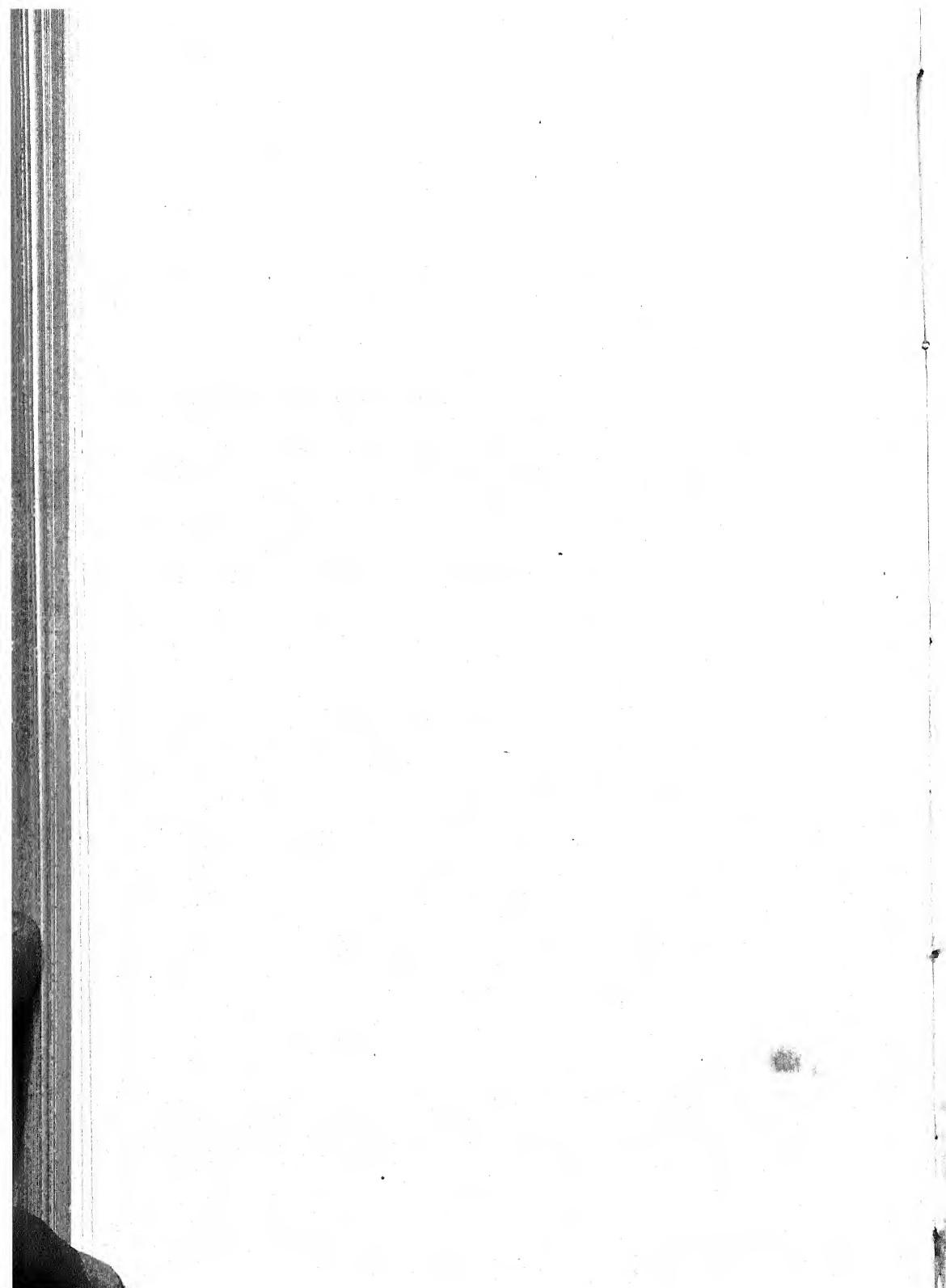
THE MEASUREMENT OF SOIL WATER [1]

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INTRODUCTION

In the field of irrigation agronomy, investigators need to have acceptable information on the status of the available soil water in the root zone. It is important to know such things as (1) the depth of the active root zone of a given crop on a given soil; (2) the water-holding capacity of the successive sections of the root zone of any given soil; and (3) the quantity of available water in each section of the root zone at any given time or, better still, the changes in that quantity from day to day at any given location.

In the course of an investigation extending over the past 4 years, it has been found that such information may be obtained by the use of the tensiometer.[2] This investigation included a long-continued series of observations on several different crops by means of tensiometers placed at successive depths in the soil at several field locations in widely separated localities. It included also the exploration of several different methods that might be used to convert tensiometric data into values of the quantity of available soil water at each of the successive soil depths at which the tensiometers are set.

The results of this investigation are reported herein.

CATEGORIES OF SOIL WATER

In considering the status of the water contained in the soil, it is convenient to recognize at least two main categories: (1) The unavailable water and (2) the available water. The unavailable water includes that portion which is held so firmly by the soil that it is not free to move as liquid water or to be absorbed by plant roots but which may be vaporized. The available water includes that portion of the total soil water which is free to move in response to gravity or capillarity or to be absorbed by plant roots. The distinction between these two categories is important because only the available water can replenish the underground supply and support plants.

The unavailable soil water comprises (1) water that, while not free to move or be absorbed by plants, may be vaporized and pass into the air; (2) water that is not vaporized under the ordinary conditions of atmospheric temperature and humidity but may be vaporized when the air temperature is raised to the boiling point of water; and

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(3) water that is held as a part of the crystal structure of the soil minerals and may be removed only by temperatures ranging above the boiling point of water. To determine the total water content of a sample of soil, the accepted procedure is to heat the sample to 105° C. in an open container and to keep it at that temperature until it ceases to lose weight. This treatment vaporizes all the available water and that portion of unavailable water in subcategories 1 and 2 but does not differentiate the available from the unavailable water.

The available water includes all the water held by the soil between its saturation point and the upper limit of the unavailable water. In current usage, the category of available soil water is subdivided into (1) the portion that moves freely in response to gravity and passes downward through a well-drained soil and (2) the portion that is held by the soil so strongly that it does not move freely in response to gravity but may move by capillarity or may be absorbed by plant roots. The upper limit of subcategory 2 is referred to as field capacity. This is not, however, a precise or even a very useful criterion, because it is influenced by time, temperature, and the conditions of subsoil drainage.

From an agronomic standpoint, it is chiefly the available soil water that merits consideration. The condition of a soil in respect to its unavailable soil water becomes of agronomic interest only when the available water has been used up or dissipated and some of the unavailable water has also been lost by vaporization. At such a time, if the soil water is replenished by rain or irrigation, a portion of the input is inactivated by restoring the deficit in the unavailable supply. The unavailable soil water may be likened to the dead storage in a water reservoir, i. e., the storage below the lowest outlet. If all the available stored water is drawn off and some of that in the dead storage is evaporated, the deficit in the dead storage must be made up from a new input before any of that input becomes available for use.

This necessary allocation of input water to restore any deficit in the unavailable supply becomes of agronomic importance with soils that hold large quantities of unavailable water. After a protracted drought during which much of the unavailable water may have been lost, a corresponding quantity of the input water must go to make up this loss and the available supply is diminished by that much.

METHODS OF MEASURING SOIL WATER

There are currently in general use three methods of measuring soil water: (1) Gravimetric, (2) electrometric, and (3) tensiometric.

GRAVIMETRIC METHOD

The gravimetric method of measuring soil water involves the collection of soil samples representing sections of the root zone. These samples are taken with a soil tube and transferred to a closed container. The container, with its moist soil, is weighed; it is then opened and placed in a drying oven maintained at 105° C. until there is no further loss in weight. The loss in weight, representing the water content, is then reported as a percentage of the weight of the dry soil.

The gravimetric method yields information as to the total water in the soil. It does not show how much of that water is available. This fact impairs the agronomic value of gravimetric findings. It is possible

to remedy this defect to some extent by determining the wilting-point range for the soils of each section of the root zone for any given location. But the gravimetric method requires the collection of many soil samples and is not practicable for observing accurately the day-to-day changes in soil water at the successive horizons of the root zone.

ELECTROMETRIC METHODS

There are several electrometric methods of measuring soil water. Of these, the one currently used involves measuring the resistance between two electrodes placed in the soil, usually embedded in a gypsum block.[3] The resistance between the electrodes varies inversely with the quantity of soil water and also with the soil temperature. The resistance method is useful in measuring soil water in the range of the wilting point. It is less sensitive to differences in the range of field capacity and above.

Other electrometric methods currently under investigation but not in general use involve measuring the thermal or the electrical capacity of the soil, both of which change as the soil water changes.

TENSIOMETRIC METHOD [4]

The tensiometric method of measuring soil water involves the use of a mercury manometer to measure the tension existing between the soil and the soil water. A porous cup is installed in the soil and connected with the manometer through a flexible copper tube of small diameter. The system is then filled with air-free (freshly boiled) water. Connection between the soil water and the water in the instrument is quickly established through the walls of the porous cup, and the tension of equilibrium is indicated by the height of the mercury column in the manometer. The manometer may be equipped with a scale the units of which are equivalent to 1 mm. of mercury or with a scale on which the smallest subdivision is equivalent to the head of 2 cm. of water; 1 mm. on the mercury scale equals 1.35 cm. on the water scale.

The scale is long enough to measure tensions up to 630 mm. of mercury, or 850 cm. of water, or approximately 0.85 atmosphere. When the tension approaches the upper limit of the scale, bubbles of air or of water vapor are likely to appear in the system. When they do, the readings may be invalid and the instrument must be serviced by refilling it with water after the tension in the soil has been reduced by rain or irrigation. The tensiometer gives precise information concerning soil-water conditions within its range, viz, from zero tension, when the soil is saturated, to approximately 0.85 atmosphere of tension, when most of the available water (80 to 90 percent) has been dissipated.

The tensiometric method appears to be by far the most accurate and the most sensitive one now in general use for measuring the available soil water within the optimum range for plants. This method as it has been used in field investigations, chiefly in irrigated areas, is the subject of the present paper.

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[3] BOUYOUCOS, G. J., and MICK, A. H. AN ELECTRICAL RESISTANCE METHOD FOR THE CONTINUOUS MEASUREMENT OF SOIL MOISTURE UNDER FIELD CONDITIONS. Mich. Agr. Expt. Sta. Tech. Bul. 172, 38 pp., illus. 1940.

[4] RICHARDS, L. A. SOIL MOISTURE TENSIOMETER MATERIALS AND CONSTRUCTION. Soil Sci. 53: 241-248, illus. 1942.

FIELD OBSERVATIONS WITH TENSIOMETERS

The investigations here reported were begun in February 1940 with the installation of four tensiometers at successive depths in the soil at the United States Rubidoux Laboratory, Riverside, Calif. The location was in a plot (plot U) allocated to citrus trees. The porous cups were placed at 6, 12, 18, and 24 inches below the ground surface, midway between two 30-year-old Valencia orange trees set 20 feet apart. Subsequently, additional instruments were installed at 36, 48, and 72 inches below the ground surface. After a time, a similar installation was made at another location in the plot so that the records from the two locations could be compared. There was good agreement between the two sets of instruments at the two locations. The observations in plot U at the Rubidoux Laboratory have been continued since 1940.

In 1941 a few tensiometers were installed at the United States Huntley Field Station, Huntley, Mont., and at the United States Yuma Field Station, Bard, Calif. Observations have been continued at these stations. In 1942 several sets of instruments were installed at the United States Scotts Bluff Field Station, Mitchell, Nebr., and observations have been continued at that station. In 1944, 2 sets of instruments with 3 or more tensiometers in each set were installed at (1) the United States Umatilla Field Station, Hermiston, Oreg.; (2) the Irrigation Branch Experiment Station, Prosser, Wash.; and (3) the United States Newlands Field Station, Fallon, Nev. Currently, tensiometric observations are reported regularly from 7 stations, with 57 instruments at 14 locations. These instruments are observed at least once a day, usually early in the morning. The tension values recorded from these observations show the day-to-day changes that occur at each of the successive horizons of the root zone.

An example of the tensiometric data obtained from an installation of seven instruments in a plot occupied by large Valencia orange trees is shown in table 1. The plot was irrigated by sprinklers during the night of May 30–31 and again during that of June 28–29. The depth of input for the tensiometer location was measured by means of eight small rain gages set close to that location.

Table 1 shows that, at the 6-inch depth, the tension rose from 245 mm. on June 18 to 568 mm. on June 28. The morning after irrigation, it stood at 3 mm. or close to saturation. From then to July 15, it rose continuously to 237 mm. A similar sequence of tension values was shown at the 12-inch depth. At the 18-inch depth, the low point of tension was not reached on the morning after irrigation. It was probably reached later that day, and the tension had started up again on the morning of June 30. At the 24-inch depth, the lowest tension probably occurred on July 1. The record of the instrument at the 36-inch depth shows that an irrigation of 0.3 foot did not contribute much water to that horizon. There was some loss of tension after July 2, but the loss was gradual and continuous until July 14. The instruments at 48 inches and at 72 inches showed that there was very little change in the soil water at those horizons during the 4 weeks of the record. It seems evident that at this location the orange trees did not draw much water from below the 36-inch horizon.

TABLE 1.—*Observed tensions at successive horizons of the soil allocated to Valencia orange trees, plot U, United States Rubidoux Laboratory, Riverside, Calif., in 1944*

Date	Tension at indicated depth (inches)						
	6	12	18	24	36	48	72
	Mm. Hg	*Mm. Hg*	*Mm. Hg*	*Mm. Hg*	*Mm. Hg*	*Mm. Hg*	*Mm. Hg*
June 18	245	218	252	482	312	205	168
June 19	272	237	271	510	320	207	170
June 20	306	257	293	528	327	208	170
June 21	352	285	316	542	330	211	172
June 22	397	313	341	553	332	211	171
June 23	454	363	375	566	339	213	174
June 24	479	398	409	571	342	213	174
June 25	511	457	451	--------	345	213	174
June 26	532	522	497	--------	350	215	175
June 27	550	578	510	515	357	217	167
June 28	568	618	514	450	367	218	170
June 29 [1]	3	9	432	538	375	221	173
June 30	50	55	84	270	372	222	172
July 1	67	73	96	106	375	223	174
July 2	78	85	107	109	381	226	175
July 3	88	95	115	114	379	227	175
July 4	98	103	123	121	370	227	176
July 5	105	110	129	123	348	225	177
July 6	115	117	135	130	323	222	175
July 7	127	127	144	138	303	219	176
July 8	136	133	148	143	280	217	175
July 9	146	139	152	148	259	212	173
July 10	155	145	156	153	243	210	172
July 11	168	153	162	158	230	205	170
July 12	181	159	168	163	225	203	170
July 13	198	168	175	170	220	203	169
July 14	210	177	179	174	206	199	168
July 15	237	187	184	181	215	198	168

[1] Input, 0.3025 foot of irrigation water during night of June 28–29.

At the United States Huntley (Mont.) Field Station, tensiometers were installed at two locations for the season of 1942: (1) In a plot of third-year alfalfa where the instruments were set at 12, 24, and 36 inches below the ground surface, and (2) in a plot of sugar beets where the instruments were set at the same depths. The soil at Huntley is a clay loam of recent alluvial origin. The subsoil contains layers of sand and gravel, which fill with water during the irrigation season. At the location of the tensiometer, an observation well that extended into a gravel stratum showed that the ground water in July stood at about 8 feet below the ground surface.

The tensiometers were set on May 20, 1942, and remained in position until September 20. The first crop of hay was cut on June 15. The data reported in table 2 relate to the period June 28 to July 25, during which the second crop of alfalfa was produced and the sugar beets were making vigorous growth. The alfalfa plot was irrigated on July 1, and the second crop of hay was cut on July 21.

The tensiometric record for the alfalfa plot shows that the plants used water from the root zone to the depth of 36 inches. The response to the irrigation of July 1 occurred sooner at 36 inches than at 12 inches, probably because this soil, when dry, shrinks to form deep cracks into which the irrigation water flows. At the 24-inch horizon, the lowest tension after the irrigation of July 1 was not reached until July 9. On July 21, when the second crop was cut, the tension at 12 inches was off the scale. At 24 inches, the tension went off the scale the next

TABLE 2.—*Observed tensions in the root zone of alfalfa and of sugar beets at the United States Huntley (Mont.) Field Station in 1942*

Date	Alfalfa, 3d year, plot K–III–3				Sugar beets, plot K–III–6			
	Water input	Tension at indicated depth (inches)			Water input	Tension at indicated depth (inches)		
		12	24	36		12	24	36
	Foot	*Mm. Hg*	*Mm. Hg*	*Mm. Hg*	*Foot*	*Mm. Hg*	*Mm. Hg*	*Mm. Hg*
June 28	0.018	557	460	264	0.018	230	70	52
June 29		570	469	268		243	67	51
June 30		580	483	306		298	71	54
July 1	.734	581	485	331	.683	28	----	----
July 2		398	470	21		37	31	22
July 3		82	437	30		53	40	27
July 4		44	394	47		66	45	30
July 5		51	316	52		82	45	32
July 6		67	257	60		102	49	34
July 7		77	210	63		122	49	37
July 8		93	195	69		150	54	40
July 9	.008	114	143	74	.008	176	54	43
July 10		136	210	77		213	55	44
July 11		162	266	81		254	57	44
July 12		200	342	90		308	61	47
July 13		241	407	97		358	63	49
July 14		284	441	105		400	66	53
July 15		332	465	114		435	70	57
July 16		376	469	120		451	68	54
July 17		434	487	137	1.008	482	73	60
July 18		483	503	162		82	38	22
July 19		519	503	182		28	40	29
July 20		544	516	216		50	45	34
July 21 [1]		(2)	520	253		64	47	36
July 22		(2)	(2)	283		84	49	38
July 23		(2)	(2)	293		108	51	40
July 24		(2)	(2)	298		130	51	43
July 25	.020	(2)	(2)	312	.020	161	58	48

[1] Alfalfa cut.　　[2] Tension off scale.

day, whereas at 36 inches it was still on the scale at the end of the period here reported. In fact, it was still on the scale (367 mm.) when the plot was irrigated on July 30.

The tensiometric record for the sugar-beet plot shows that the root zone of this crop was not as deep as that of alfalfa on this Huntley soil. The instruments at 24 and at 36 inches showed very little change of tension through the period here reported. In fact, through the whole season the highest tension observed at the 24-inch horizon was 83 mm. on August 25, when the instrument at 12 inches had been off the scale for 3 days and when the one at 36 inches also reached its highest tension (70 mm.).

The day-to-day conditions of tension in these two plots are shown in figure 1. The record, as charted in these graphs, extends from early June to mid-August 1942 and thus includes the period covered by the data in table 2. These graphs show that the alfalfa crop drew water freely from the root zone to the depth of 36 inches at least, whereas the sugar beets did not draw much water from the 24-inch horizon or below. One obvious consequence of this difference between the depths of the root zones of these crops is that the sugar beets had to be irrigated four times during the season, whereas the alfalfa was irrigated only twice. The sugar beets made normal, vigorous growth
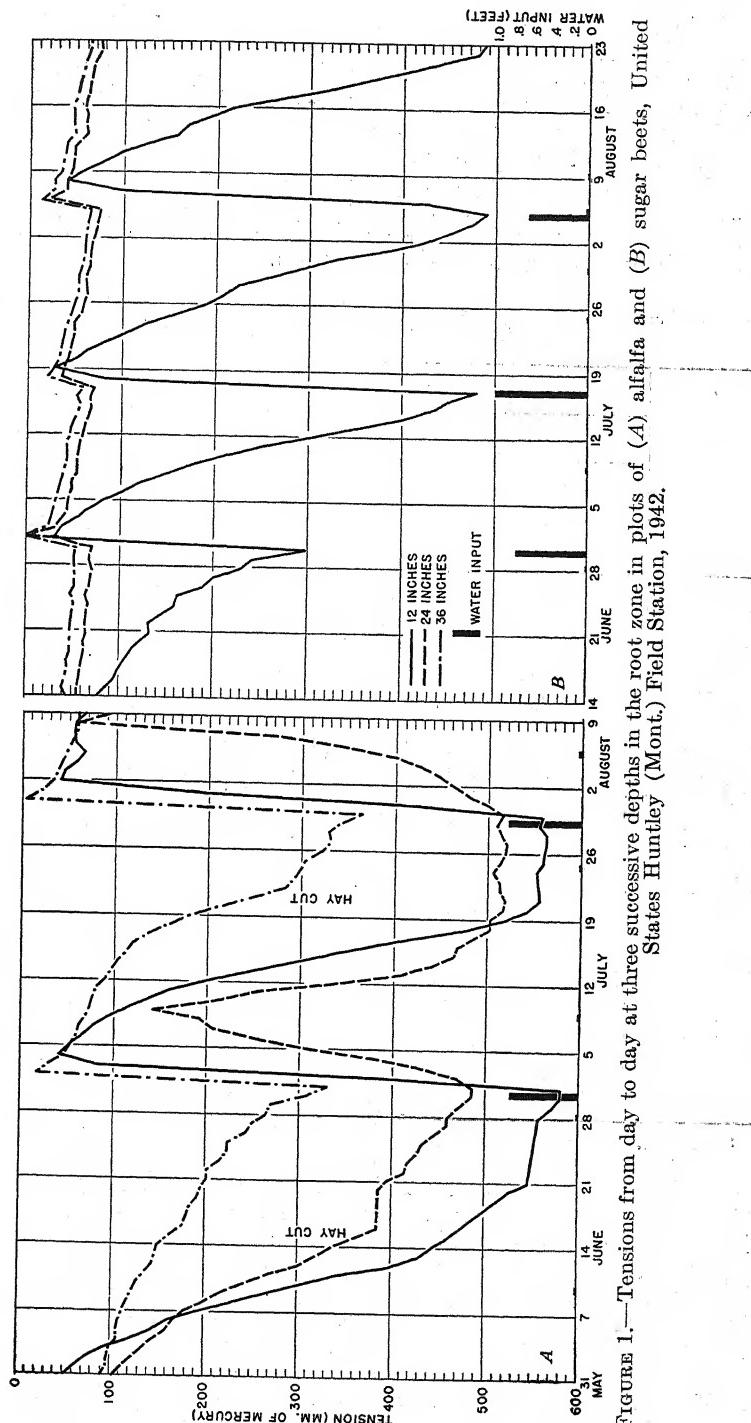
FIGURE 1.—Tensions from day to day at three successive depths in the root zone in plots of (*A*) alfalfa and (*B*) sugar beets, United States Huntley (Mont.) Field Station, 1942.

and yielded at the rate of 14.3 tons per acre. The alfalfa also grew well and yielded for three crops at the rate of 4.3 tons per acre.

The findings of 1942, that the active root zone for sugar beets was less than 24 inches deep and that the roots of alfalfa drew water from 36 inches and possibly below, led to a different series of settings in 1943. For that season, five instruments were set in the alfalfa plot at 6, 12, 24, 48, and 60 inches. The tensiometric data from this installation are shown in table 3. This record shows that the alfalfa roots drew some water from the 48-inch horizon but probably did not draw much, if any, from the 60-inch horizon.

TABLE 3.—*Observed tensions in the root zone of alfalfa and of sugar beets at the United States Huntley (Mont.) Field Station in 1943*

	Plot allocated to—									
	Alfalfa, 3d year, plot K–III–2					Sugar beets, plot K–III–5				
Date	Water input	Tension at indicated depth (inches)					Water input	Tension at indicated depth (inches)			
		6	12	24	48	60		6	12	18	24
	Foot	*Mm.Hg*	*Mm.Hg*	*Mm.Hg*	*Mm.Hg*	*Mm.Hg*	*Foot*	*Mm.Hg*	*Mm.Hg*	*Mm.Hg*	*Mm.Hg*
July 12	-------	391	532	172	162	90	-------	540	125	110	95
July 13	-------		542	178	170	101	-------		173	111	100
July 14	-------	572	543	180	175	105	-------		202	115	100
July 15	-------	567	542	178	164	116	0. 992	520	235	119	104
July 16	0. 525	574	547	180	165	110	-------	22	2	22	10
July 17	-------	25	22	29	190	105	-------	40	28	50	43
July 18	-------	44	45	40	190	107	-------	61	53	60	50
July 19	-------	69	66	52	163	92	-------	79	66	64	57
July 20	-------	142	95	65	68	82	-------	105	80	70	65
July 21	-------	236	125	80	82	84	-------	148	97	75	65
July 22	. 031	449	166	93	86	84	. 031	220	107	79	70
July 23	-------	67	214	95	96	79	-------	161	63	75	69
July 24	-------	129	272	80	100	77	-------	224	125	87	75
July 25	-------	369	357	60	100	82	-------	259	142	101	83
July 26	-------	523	435	68	110	84	-------	406	164	105	92
July 27	. 003	566	482	75	120	95	. 003	420	173	107	84
July 28	-------	571	500	96	118	95	-------	461	164	102	88
July 29 [1]	-------	576	514	93	126	92	-------	469	213	112	94
July 30	-------	575	516	95	133	94	. 716	479	224	137	100
July 31	-------	569	515	95	130	92	-------	15	15	14	14
Aug. 1	-------	544	502	91	110	87	-------	38	15	51	45
Aug. 2	. 041	396	357	97	86	94	. 041	57	29	62	50
Aug. 3	-------	24	439	99	118	88	-------	22	22	27	51
Aug. 4	. 019	77	485	104	128	90	. 019	39	29	62	55
Aug. 5	-------	27	507	107	130	90	-------	27	26	61	58
Aug. 6	-------	101	520	113	131	90	-------	52	33	71	61
Aug. 7	-------	309	527	111	134	92	-------	83	37	75	65
Aug. 8	-------	459	533	120	137	92	-------	125	46	83	72

[1] Alfalfa cut.

In the sugar-beet plot for the season of 1943 there were four tensiometers, set at 6, 12, 18, and 24 inches. The observations recorded in table 3 confirm the findings of 1942 for the 24-inch horizon, viz, that the sugar beets did not draw much water from that horizon. The highest tension reported for that depth in table 3 is 104 mm. on July 15, when the tension at the 6-inch horizon had been near the top of the scale for 4 days. The highest tension (115 mm.) during the whole season, for the 24-inch horizon, was on August 14, at which time the tension at 6 inches had been near the top of the scale for several days.

The range of tensions at the 18-inch horizon show that the sugar-beet roots were active to that depth in the Huntley soil.

At the United States Yuma Field Station, Bard, Calif., two sets of tensiometers were installed in plots allocated to wheat and to barley. These instruments were placed in the ground in December 1942 soon after the crops were seeded. They remained in place until late in the following April, when the crops were harvested. The soil at this station is sandy, with an admixture of river silt in the surface layer to the depth of 12 to 14 inches. The subsoil is sandy, with ground water at the depth of 5 to 6 feet. The instruments were installed in each plot at 7, 19, and 31 inches below the ground surface. The plots were nearly level; each was surrounded by a low border and irrigated by flooding.

TABLE 4.—*Observed tensions in the root zone of wheat and of barley at the United States Yuma Field Station, Bard, Calif., in 1943*

	Plot allocated to—							
	Wheat, plot C–I–15				Barley, plot C–II–15			
Date	Water input	Tension at indicated depth (inches)			Water input	Tension at indicated depth (inches)		
		7	19	31		7	19	31
	Foot	*Mm. Hg*	*Mm. Hg*	*Mm. Hg*	*Foot*	*Mm. Hg*	*Mm. Hg*	*Mm. Hg*
Mar. 7		(1)	124	72		(1)	156	85
Mar. 8	0.461	(1)	159	71	0.498	(1)	183	86
Mar. 9		33	41	46		279	78	63
Mar. 10		59	42	43		74	64	59
Mar. 11		80	51	47		93	62	58
Mar. 12		96	54	51		107	60	59
Mar. 13		122	56	53		129	62	61
Mar. 14		175	60	55		175	67	64
Mar. 15		305	62	56		257	68	65
Mar. 16		471	65	58		386	71	67
Mar. 17		565	68	60		492	74	70
Mar. 18		602	70	61		558	78	71
Mar. 19		(1)	75	63		604	84	74
Mar. 20		(1)	79	64		(1)	97	75
Mar. 21		(1)	83	65		(1)	136	78
Mar. 22		(1)	86	65		(1)	161	80
Mar. 23		(1)	95	65		(1)	204	81
Mar. 24		(1)	138	66		(1)	288	83
Mar. 25		(1)	195	67		(1)	315	85
Mar. 26		(1)	283	69		(1)	354	88
Mar. 27		(1)	332	70		(1)	378	90
Mar. 28		(1)	378	70			394	92
Mar. 29	.484	(1)	431	69	.555	(1)	407	94
Mar. 30		46	37	52		146	90	45
Mar. 31		71	46	48		66	58	50
Apr. 1		90	47	49		70	57	56
Apr. 2		110	53	50		82	66	60
Apr. 3		139	70	52		102	69	62

[1] Tension off scale.

The records for the two sets of tensiometers for 4 weeks ending April 3, 1943, are shown in table 4. Both plots had been irrigated on February 15. When the tabulated record began (March 7) both the instruments at 7 inches were off the scale. The tensions shown by the instruments at 19 inches indicated that the crops had begun to draw water from that horizon. Prior to the period covered by the

table there had been no evidence of the use of water from the 19-inch horizon, even though both the instruments at 7 inches had been off the scale prior to the irrigation of February 15. After the irrigation of March 8, the instruments at 7 inches showed a rapid increase of tension, reaching the top of the scale on March 19 and 20. The instruments at 19 inches showed relatively little increase in tension until after those at 7 inches had gone off the scale. Then they began a rapid rise, which continued until the next irrigation. The instruments at 31 inches showed very little change in tension throughout the period of the tabulated record. In fact, the complete record for the whole crop period, from December 19 to April 24, shows that the highest tension at the 31-inch horizon was 94 mm. This seems convincing evidence that the lower limit of the active root zone of these cereal crops in the Yuma soil was well above 31 inches.

At the Yuma Field Station, in the summer of 1943, tensiometers were installed in two plots of the Acala variety of cotton. The instruments were set at depths of 7, 19, and 31 inches. The cotton was planted the latter part of March, and the instruments were installed on May 1, when the plants were about 6 inches tall. The first flowers opened on June 3, and the first open boll was observed on July 20. The tabulated record (table 5) covers the 4 weeks from August 22, when the plants were fully grown and were fruiting heavily. This probably includes the period of the greatest use of water by the plants. It has been learned through long experience at the Yuma Field Station that cotton must be irrigated frequently if good yields are to be obtained. Irrigation water is usually available only once a week. After the first of July, if an irrigation is missed, the plants wilt in the afternoon after about 10 days. For example, plot C–I–21 was irrigated on June 29. It was not irrigated again until July 13, and the plants began to wilt on the afternoon of July 10. Plot C–II–16 was irrigated July 5, and the plants began to wilt on July 11 and wilted again each afternoon until the plot was irrigated on July 13. When these first wiltings occurred, the tensiometers at 7 inches were off the scale, but the instruments at 19 inches showed that the tensions were 57 mm. in plot C–I–21 and 78 mm. in plot C–II–16.

During the period of the records shown in table 5, the plots were irrigated each week with one exception. The record for each plot shows that the tension at the 7-inch horizon increased rapidly after each irrigation, whereas the increase was very small at both the 19-inch and the 31-inch horizons. In this respect, the data of the table are representative of the data for the whole season. Plot C–I–21 was not irrigated on September 13, after 1 week. The record shows that the manometer reached the top of the scale on September 15. It remained off the scale until the plot was irrigated on September 20, yet the continued low tensions at the 19-inch horizon indicate that the plants did not draw much water from that depth.

These tensiometric records, including the records for the whole season of 1943 and records from two other plots of cotton in 1944, show that on the Yuma station soil the active root zone of cotton was less than 19 inches deep. This finding makes it clear why it is necessary at that station to irrigate cotton 14 to 15 times during the season if good yields are to be obtained.

TABLE 5.—*Observed tensions in the root zone of cotton at the United States Yuma Field Station, Bard, Calif., in 1943*

Date	Cotton, plot C–I–21				Cotton, plot C–II–16			
	Water input	Tension at indicated depth (inches)			Water input	Tension at indicated depth (inches)		
		7	19	31		7	19	31
	Foot	*Mm. Hg*	*Mm. Hg*	*Mm. Hg*	*Foot*	*Mm. Hg*	*Mm. Hg*	*Mm. Hg*
Aug. 22		415	78	60		498	75	63
Aug. 23	0.205	535	86	65	0.199	538	78	67
Aug. 24		40	48	43		38	44	62
Aug. 25		76	52	44		70	53	62
Aug. 26		108	56	50		105	60	61
Aug. 27		170	58	53		157	66	62
Aug. 28		322	60	56		278	70	65
Aug. 29		448	61	60		476	74	66
Aug. 30	.255	536	62	61	.205	574	77	67
Aug. 31		38	36	42		37	43	65
Sept. 1		70	42	47		62	52	63
Sept. 2		102	45	50		99	59	63
Sept. 3		171	48	55		151	65	63
Sept. 4		263	52	57		235	68	64
Sept. 5		372	53	60		387	73	65
Sept. 6	.273	456	56	63	.225	535	76	67
Sept. 7		30	25	46		41	41	60
Sept. 8		61	38	52		61	52	56
Sept. 9		91	41	55		93	60	57
Sept. 10		138	45	56		130	64	58
Sept. 11		211	49	61		190	67	60
Sept. 12		310	52	65		298	71	63
Sept. 13		395	54	66	.210	438	74	64
Sept. 14		468	55	67		32	42	57
Sept. 15		516	57	70		58	52	55
Sept. 16		(¹)	60	72		86	58	56
Sept. 17		(¹)	62	74		120	63	57
Sept. 18		(¹)	66	77		174	66	60

¹ Tension off scale.

At the United States Scotts Bluff (Nebr.) Field Station, it was found by tensiometric observations during the season of 1942 that alfalfa used water freely to and including the 36-inch horizon. Because of this finding, the instruments installed in an alfalfa plot in 1943 were set at 12, 24, 48, and 72 inches. The soil here is classed as a very fine sandy loam. There is no ground-water table in proximity to the root zone. When the instruments were installed on May 26, the soil was dry to 6 feet or more and all the manometers rose nearly to the top of the scale. There were frequent showers between May 30 and June 16, and the plot was irrigated on June 10. These water inputs so reduced the tension that on July 1 the tension was 293 mm. at 12 inches, 258 mm. at 24 inches, 230 mm. at 48 inches, and 160 mm. at 72 inches. There was no effective rain for several weeks after July 1, and the tensions continued to rise at all four horizons until the plot was irrigated on July 20.

The tensiometric record for this alfalfa plot (table 6) for the 4 weeks beginning July 18 shows that the irrigation input of July 20 penetrated to the 48-inch horizon the following day and reached the 72-inch horizon about a week later. After the irrigation of July 20, the tensions rose rapidly at the 12-inch and 24-inch horizons and more slowly at the 48-inch horizon. There was little change at 72 inches.

After the period of the tabulated record the upward trend of tensions continued, notwithstanding a rain of 0.44 inch on August 20. By August 28 all the manometers, except that at 72 inches, were off the scale. The reading at 72 inches was 222 mm.

TABLE 6.—*Observed tensions in the root zone of alfalfa and of potatoes at the United States Scotts Bluff (Nebr.) Field Station in 1943*

	Plot allocated to—								
	Alfalfa, 2d year, plot K–I–10					Potatoes, plot K–I–11			
Date	Water input	Tension at indicated depth (inches)				Water input	Tension at indicated depth (inches)			
		12	24	48	72		12	18	24	36
	Foot	*Mm. Hg*	*Mm. Hg*	*Mm. Hg*	*Mm. Hg*	*Foot*	*Mm. Hg*	*Mm. Hg*	*Mm. Hg*	*Mm. Hg*
July 18	--------	447	508	455	244	--------	278	260	185	447
July 19	--------	456	473	464	257	--------	297	276	193	452
July 20	0.715	453	453	464	262	--------	320	273	193	451
July 21	--------	77	73	268	262	--------	346	280	103	452
July 22	.009	92	84	--------	270	0.009	363	255	--------	--------
July 23	.012	97	93	321	275	.559	331	260	172	--------
July 24	--------	107	98	428	277	--------	92	85	75	--------
July 25	--------	115	107	450	282	--------	109	104	83	431
July 26	--------	122	115	462	277	--------	121	114	91	480
July 27 [1]	--------	130	125	467	276	--------	132	124	101	450
July 28	--------	141	136	457	273	--------	143	131	107	450
July 29	--------	131	135	418	--------	--------	154	139	112	358
July 30	--------	139	141	453	--------	--------	167	147	122	380
July 31	--------	145	146	455	197	--------	183	153	128	355
Aug. 1	--------	152	154	454	201	--------	205	161	136	331
Aug. 2	--------	161	162	454	202	--------	232	170	149	317
Aug. 3	--------	170	171	405	208	--------	257	170	159	311
Aug. 4	--------	183	184	434	208	.247	306	187	176	307
Aug. 5	--------	199	200	443	205	--------	120	124	190	307
Aug. 6	--------	217	213	447	196	--------	125	119	153	308
Aug. 7	--------	248	239	455	191	--------	147	130	150	309
Aug. 8	--------	286	272	446	185	--------	169	140	153	304
Aug. 9	--------	354	317	453	190	--------	197	156	173	312
Aug. 10	--------	417	390	444	187	--------	233	169	194	298
Aug. 11	--------	465	455	441	182	--------	288	188	220	298
Aug. 12	--------	479	481	443	187	--------	354	207	253	303
Aug. 13	--------	507	492	439	190	--------	419	231	278	307
Aug. 14	--------	499	506	456	191	--------	415	281	316	316

[1] Alfalfa cut.

The alfalfa plot was irrigated on August 31 with 1.006 feet of water. This input reduced the tension immediately down to the 48-inch horizon and reached the 72-inch horizon on September 3. This review of the seasonal record of tensions shows that at Scotts Bluff the active root zone of alfalfa was deeper than 48 inches but probably did not extend to 72 inches.

The investigations at Scotts Bluff in 1943 included also an installation of four tensiometers in a plot allocated to potatoes. The instruments were set at 12, 18, 24, and 36 inches on July 3. The potatoes had been planted on June 12 and had emerged on June 29. The soil was dry when the instruments were set, particularly so at the 36-inch horizon. The instrument at 24 inches did not function well at first, and its record prior to the irrigation of July 23 may not be valid. The irrigation of that date (0.559 foot) penetrated immediately to 24 inches and reached the 36-inch horizon within a week. The light irrigation (0.247 foot) on August 4 penetrated to 18 inches, with

slight effect at 24 inches but none at 36 inches. The record for the whole season indicates that the active root zone of potatoes at Scotts Bluff extended to 24 inches or more but probably did not extend to 36 inches.

These examples of the tensiometric records from locations having a variety of different soils and a number of different crops show that the tensiometer makes available useful information regarding the depth of the root zone and the day-to-day changes that occur in the available soil water. The usefulness of such information is not confined to irrigated areas but is particularly valuable in any situation where irrigation water can be used when it is needed. In connection with agronomic investigations of many different sorts, it would be helpful to know from day to day the status of the available soil water. The observed differences of behavior between crop species or varieties may be associated with ranges in the depth of the root zone and consequently with the quantity of available soil water. The effects of differences in tillage practice or in weed control may be evaluated by observing the resulting differences in the quantity of available soil water. Moreover, in fertilizer experiments the crop yields often are so limited by the lack of available soil water at critical periods that the effects of the fertilizer treatments are not fully expressed.

QUANTITATIVE INTERPRETATION OF TENSIOMETRIC DATA

The measurement of the tension existing between the soil and its water yields useful agronomic information. But the question immediately arises: What does a given tension value mean in terms of the quantity of available soil water? It should be clearly understood that tension values are related only to the conditions of available soil water. The unavailable soil water, whether its quantity be large or small, has no effect on tension values.

There is an inverse relation between tension values and the quantities of available soil water. In other words, there is no measurable tension existing in a soil that is saturated with water. As the quantity of soil water decreases from the condition of saturation the tension increases. Thus it is obviously possible to establish the relation between the observed tension and the quantity of soil water by the simple expedient of determining, for any soil, the quantities of soil water that are associated with a series of observed tensions.

Exploration of this field of inquiry shows at once that, for any given soil, the tension value associated with any given quantity of available soil water is influenced by the apparent density of the soil. Thus, for any given soil with any given quantity of available water, the tension values decrease as the apparent density increases or as the soil becomes more compact. It is equally true that the apparent density of the soil is similarly related to the quantity of water required to saturate it. That is to say, as the soil becomes more compact less water is required to saturate it.

This relation between tension and apparent-density values makes it essential that in establishing, for field use, the relation between tension values and soil-water values, the sample of soil used should have the same apparent density and if possible the same "structure" as in the field.

In the investigations here reported, the objective has been to determine, for the soil of each location where tensiometers have been installed in the field, the relation between observed tension values and the quantity of available soil water. In the course of the investigation, three different procedures have been used: (1) The method of field sampling, (2) that of using soil cores in closed containers, and (3) that of using open containers with plants growing in the soil.

METHOD OF FIELD SAMPLING

By the method of field sampling, a number of tensiometers were set in a field and the tension values were observed and recorded daily. From time to time the soil was removed from the vicinity of one of the tensiometers to make it possible to obtain several samples from the soil immediately adjacent to the porous cup. On these soil samples, eight or more in number, the water content was determined by drying the soil to constant weight at 105° C., and the mean value for the soil water so determined was compared with the tension value observed just before the samples were taken. In the course of time, it was possible to obtain soil-water values corresponding to a fairly wide range of tension values.

The data of table 7 show the findings from a series of 24 such observations made in the plot at the Rubidoux Laboratory, in which there had been an installation of tensiometers since 1940. The entries are arranged in the order of the soil-water values, which range from 16.2 to 5.7 percent. The associated tension values range from 21 to 494 mm. The data of the table are shown also in figure 2. It is obvious, from both the table and the graph, that the points do not fall into a narrow path, but there is evidently an inverse relation between tension and soil water.

TABLE 7.—*Tension and soil-water values based on field sampling, plot U, Rubidoux Laboratory, December 1942 to April 1943*

Date	Tensiometer		Soil water	Date	Tensiometer		Soil water
	Depth	Reading			Depth	Reading	
	Inches	*Mm. Hg*	*Percent* [1]		*Inches*	*Mm. Hg*	*Percent* [1]
Jan. 13	12	21	16.2	Feb. 3	36	118	10.9
Jan. 16	12	58	14.4	Feb. 27	12	83	10.3
Jan. 14	12	40	14.2	Jan. 21	12	115	10.2
Jan. 29	12	51	13.8	Apr. 5	12	150	9.7
Jan. 18	12	68	13.7	Dec. 28	12	185	8.8
Jan. 29	12	78	13.4	Apr. 23	12	186	8.7
Feb. 26	12	45	13.4	Dec. 17	12	178	8.4
Feb. 26	12	54	12.8	Dec. 28	12	239	8.1
Feb. 27	12	63	12.0	Jan. 11	12	289	7.1
Jan. 18	12	88	11.9	Jan. 11	12	369	7.0
Feb. 27	12	67	11.7	Jan. 8	12	362	6.5
Jan. 18	12	65	11.6	Jan. 11	12	494	5.7

[1] Gravimetric.

While this method of field sampling affords direct information on the relation between tension and soil water, it has the following disadvantages: (1) It is not easy to obtain soil samples when the tension is at or close to zero; (2) it may take a long time to obtain representative samples related to a wide range of tensions; (3) slight differences in the character (particle size) of the soil at the different

locations or horizons may influence the quantity of total soil water but have much less influence on the quantity of available soil water, which is the part that influences the tension.

METHOD OF SOIL CORES

The method of soil cores in closed containers involved the use of steel cylinders, some 4 inches, and one 5 inches, in diameter and 5 to 6 inches long, fitted with tight covers. At the selected location and
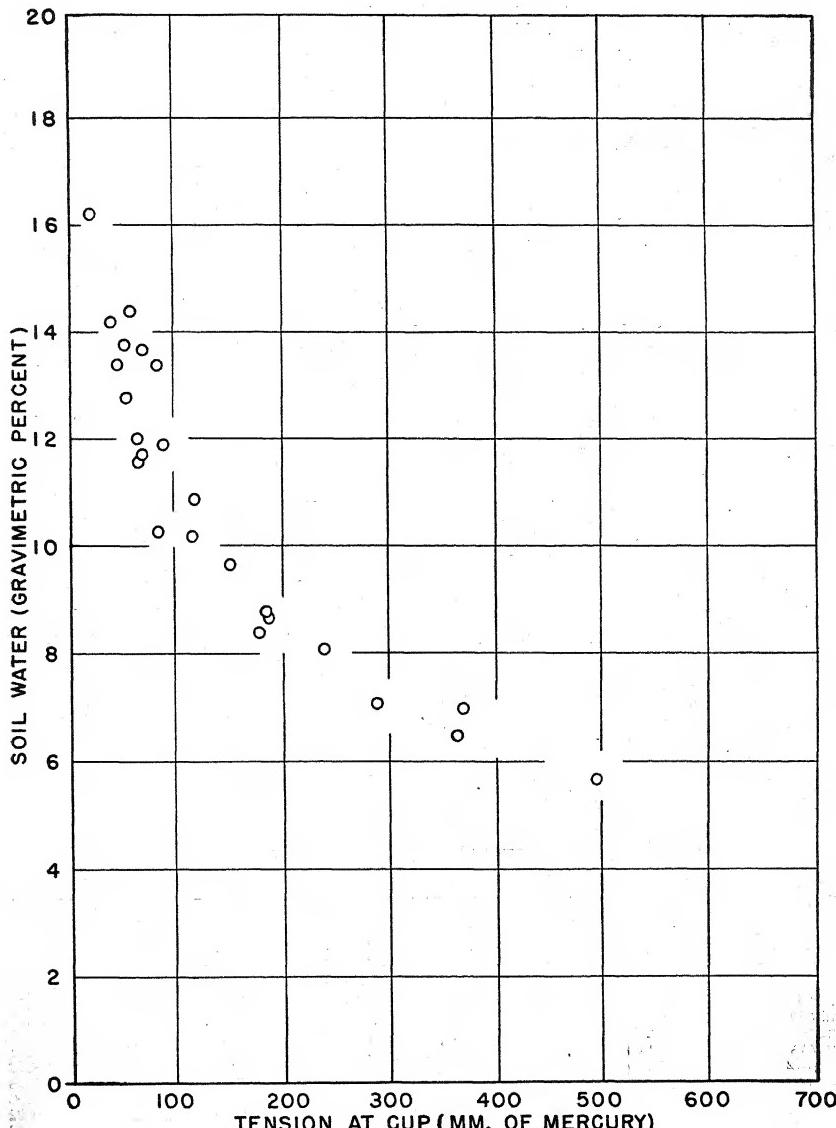
FIGURE 2.—The relation between tension and soil water as determined by the method of field sampling, plot U, Rubidoux Laboratory, Riverside, Calif.

depth, the cylinder was forced into the soil until the core of soil protruded above the top of the cylinder. Then the cylinder, with its soil core, was taken up; the core was cut off flush with the ends of the cylinder; and the metal covers, fitted with gaskets, were fastened in place. A tensiometer was installed with its porous cup near the center of the soil core. The whole system was weighed each day, and the tension was observed. When tensiometric equilibrium was attained, as indicated by no change in tension from day to day, a measured quantity of water was added and the resulting change in tension was observed. When equilibrium of tension was again attained, more water was added. This process was continued until the soil approached saturation and the tension stood at zero or slightly above. This completed the sorption run.

The soil was then aerated by drawing a stream of dry air through it to remove a portion of the water. The aeration apertures were then closed, and the tension was observed each day until it remained constant for several days. Then another portion of the soil water was removed by aeration. This process was continued until the tension reached equilibrium near the upper end of its measurable range. This completed the desorption run.

Each soil core was put through one or more cycles, i. e., a sorption run and a desorption run, after which the apparatus was taken down and the soil was dried to determine its mass. It was then possible to compute the successive percentages of soil water associated with the equilibrium tensions.

The data in table 8 show the successive values for the equilibrium tension and the soil water observed with a 'core of soil from near the tensiometer location in the orchard plot at the Rubidoux Laboratory. Observations on this core of soil were begun on April 8, 1943. During the first month, the core was aerated and dried down to 5.94 percent of water before the sorption run was started. The sorption run was continued until two successive equilibrium tensions of 18 mm. were observed. It has been found difficult with the soil cores to attain satisfactory equilibrium tensions at zero with diurnal changes in temperature. Upon completion of the sorption run, the soil was aerated by a method that permitted the removal of a known quantity of water each time. This desorption run was continued until an

TABLE 8.—*Tension and soil-water values obtained by the use of a closed container with a core of soil 9 to 15 inches in depth from plot U, Rubidoux Laboratory, experiment 59–5, June 1943 to January 1944*

Date	Tension	Soil water		Date	Tension	Soil water	
	Mm. Hg	*Grams*	*Percent*		*Mm. Hg*	*Grams*	*Percent*
June 10	242	603	7. 38	Sept. 22	75	1,174	14. 37
June 18	146	722	8. 84	Sept. 29	87	1,118	13. 68
June 23	100	840	10. 28	Oct. 7	93	1,082	13. 24
July 1	73	961	11. 76	Oct. 12	103	1,028	12. 58
July 5	52	1,078	13. 19	Oct. 20	111	963	11. 79
July 13	40	1,203	14. 72	Oct. 26	126	898	10. 99
July 21	29	1,324	16. 21	Nov. 4	153	810	9. 91
July 28	18	1,448	17. 72	Nov. 10	177	754	9. 23
Aug. 12	18	1,508	18. 46	Nov. 18	246	664	8. 13
Aug. 20	26	1,470	17. 99	Dec. 2	343	595	7. 28
Aug. 27	32	1,440	17. 63	Dec. 14	491	531	6. 50
Sept. 2	37	1,406	17. 21	Dec. 27	624	492	6. 02
Sept. 9	52	1,312	16. 06	Jan. 10	469	523	6. 40
Sept. 15	65	1,241	15. 19	Jan. 20	365	553	6. 77

equilibrium tension of 624 mm. was attained when the soil water was at 6.02 percent, after which water was added twice in small quantities to obtain points on the sorption path below those of the first run.

The data of table 8 are plotted in figure 3. From this graph it will be seen that for any given soil-water value the tension value is lower for the sorption run than for the desorption run. Such differences have been observed with all the soil cores used in these investi-
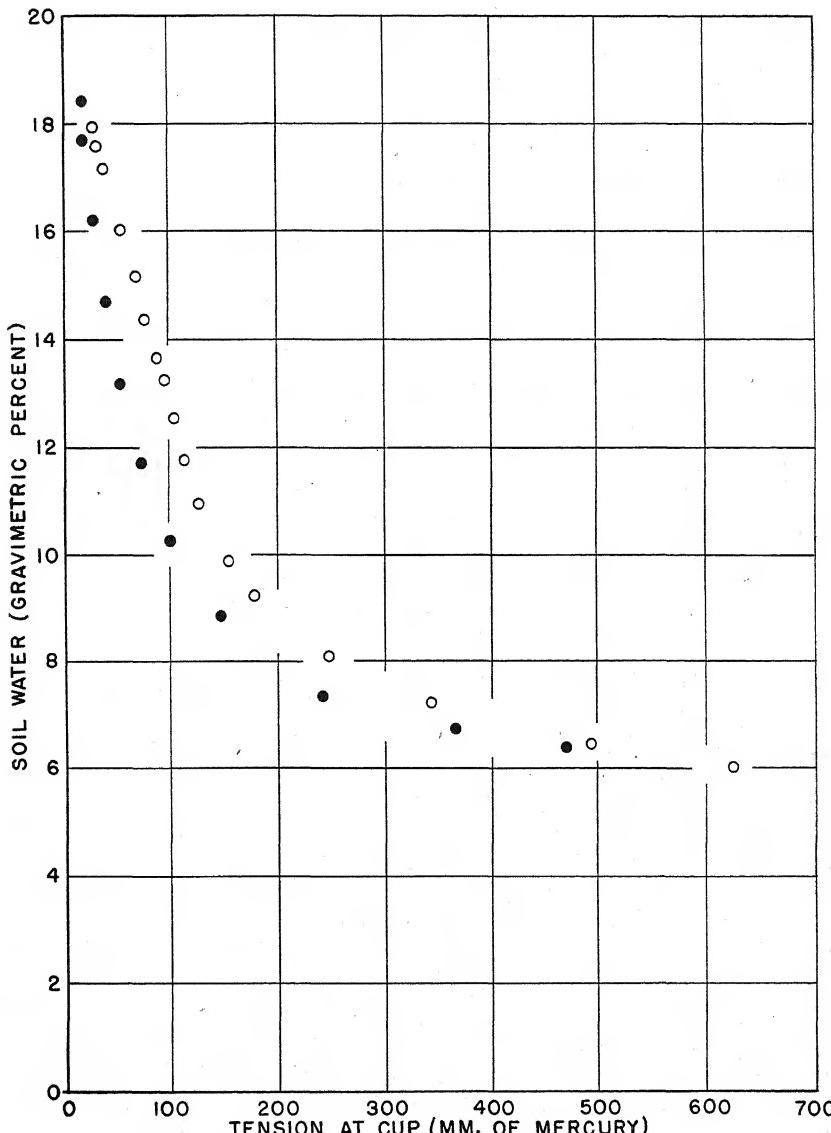
FIGURE 3.—The successive points of the sorption run (black circles) with a soil core in a closed container and of the desorption run (open circles) on the same core. Plot U, depth 9–15 inches, Rubidoux Laboratory, Riverside, Calif.

gations. Whether these differences are due to failure to attain true equilibrium between changes in soil water or to inherent characteristics of the soil has not been determined.

The methods involving the closed container yield a series of points that are acceptably consistent not only for each core but also between several cores from the same location. But the fact that the point path of the sorption run does not coincide with that of the desorption run makes for uncertainty as to which of the two is the more acceptable. The method has two other disadvantages: (1) It takes several months to complete a full cycle of observations, and (2) there is uncertainty as to when the condition of saturation has been attained.

The data here reported for the soil core in the closed container may be compared with those reported for the method of field sampling (table 7 and fig. 2). This comparison is justified because both experiments involved soil from the same plot. It will be seen that, considering the random nature of the observations, the agreement is good.

METHOD OF OPEN CONTAINERS

With the method employing open containers with plants growing in the soil, several different sizes of containers were used. The largest were iron pails 32 cm. deep by 28.5 cm. in diameter, with a volume capacity, to 3 cm. below the rim, of 18.5 liters, or of 27 to 28 kg. of dry soil. The smallest containers were 25 cm. in diameter and deep enough to hold 10 cm. of soil to 2.5 cm. below the rim; these held 7 to 8 kg. of dry soil. The container was provided with an input tube of small diameter, discharging at the center of the bottom, to the outer end of which a glass tube with stopcock was attached to control the water input and to serve as an indicator of the water level in the saturated soil. In the earlier experiments, a layer of gravel was placed in the bottom of the container to aid the distribution of water. This proved to be a source of error because of the free water held in the gravel during the early part of a run.

A tensiometer support was attached to the side wall of the container with the porous cup placed near the center of the soil mass (fig. 4). When the soil was placed in the container, its mass and water content were determined, it was packed to approximately the apparent density of its field condition, and its volume was subsequently measured from time to time as a check on changes in apparent density. The several components of the apparatus were weighed, so that by weighing the whole system from day to day the quantity of soil water could be computed. Seedling plants of alfalfa, grass, or clover were started in the soil when the apparatus was set up. The water was added either from below through the input tube or by surface applications. Usually, water was added until the soil was saturated and the observed tension was at or near zero; it was then withheld until the plants had used all or nearly all the available water. The period required to exhaust the available water is referred to as a run.

This method was found the most acceptable of the 3 methods investigated. It is not free from certain idiosyncrasies due in part to effects of temperature changes in the soil and in part to the position of the porous cup of the tensiometer in the soil in relation to the zone of most active root absorption. But after an apparatus has been set up and the plants are well grown, a run may be made in a week or 10 days

and repeated often enough to afford an acceptable assemblage of data and opportunity to observe the nature and extent of the changes in the relation between tension and soil-water values that occur with the evolution of soil structure consequent upon the repeated wetting and drying and the action of plant roots. More than 20 consecutive runs have been made with some soil samples, and it has been found
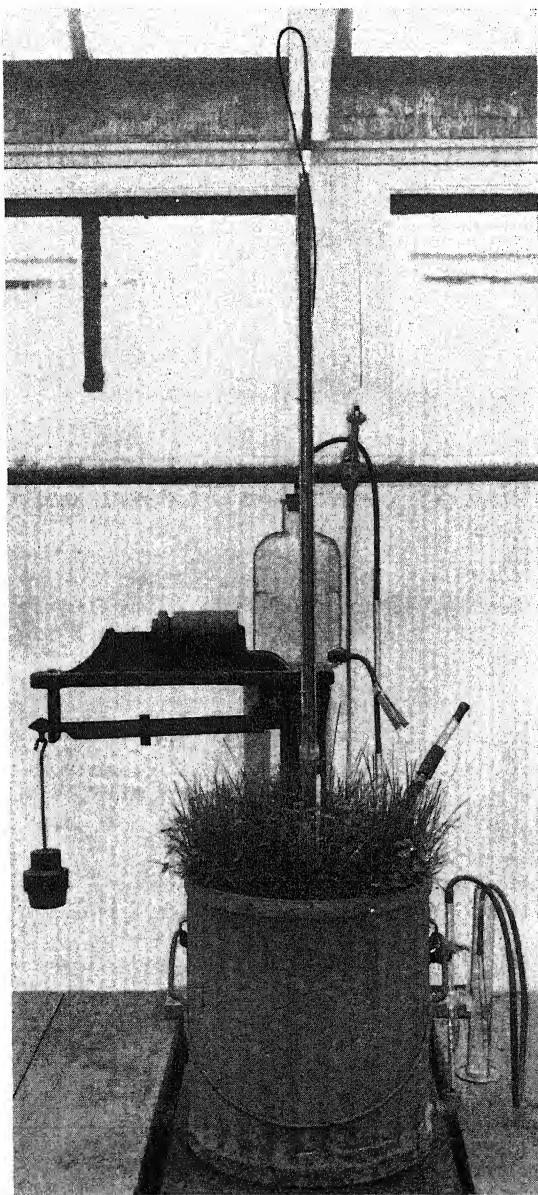
FIGURE 4.—A container used for the tensiometric calibration of soil.

that after the first few runs there is good agreement among the successive point paths.

The findings on one soil, as calibrated in an open container, are shown in table 9 and illustrated in figure 5. There were 24 successive runs made with this soil. Several of these runs were continued beyond the range of the tensiometer until the plants wilted. Wilting occurred at various levels of soil water, depending upon the rate at which water
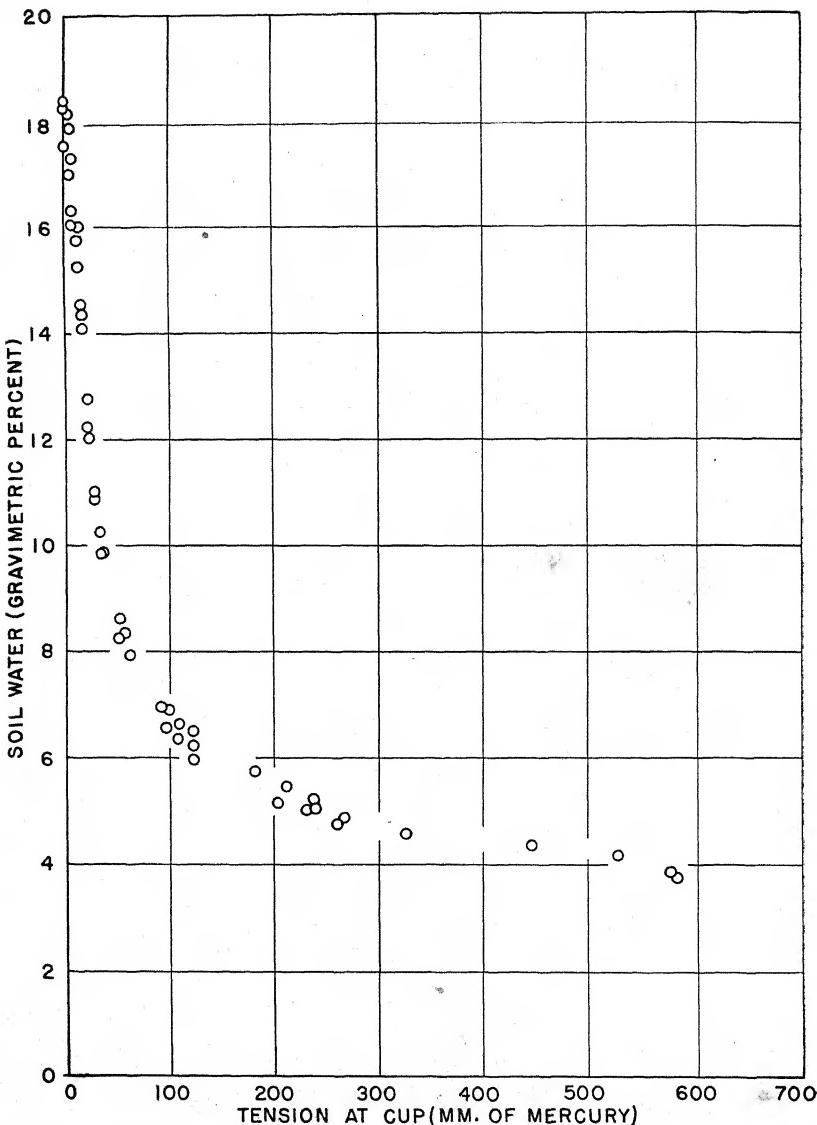
FIGURE 5.—The relation between tension and soil water with Lanham (Md.) soil, observed during three successive runs, Nos. 22–24, in an open container with perennial ryegrass and white clover.

was used by the plants. The range of soil-water percentages at the onset of wilting in 6 of the runs was from 3.20 to 3.86.

It will be seen in figure 5 that the points for the three runs fell into a narrow path forming a curve. This curve starts at approximately 18 percent and zero tension and ends at approximately 4 percent at the highest measurable tension. After the soil from a given field location in which tensiometers were installed has been calibrated, the curve that best fits the plotted point path serves as a frame of reference for converting observed tension values into the corresponding soil-water values.

TABLE 9.—*Tension and soil-water values for 3 successive runs, Nos. 22–24, Lanham (Md.) soil, February 5 to March 18, 1944*

Run 22		Run 23		Run 24	
Tension	Soil water	Tension	Soil water	Tension	Soil water
Mm. Hg	*Percent*	*Mm. Hg*	*Percent*	*Mm. Hg*	*Percent*
0	18.49	0	17.54	0	18.35
5	18.20	6	17.03	6	17.91
8	17.32	10	16.07	9	17.32
14	15.78	13	15.27	14	16.00
17	14.54	17	14.10	18	14.32
28	11.03	21	12.27	21	12.78
36	9.89	26	10.88	23	12.05
56	8.32	33	9.86	33	10.26
97	6.93	50	8.25	38	9.57
120	6.49	96	6.56	50	8.61
181	5.76	106	6.34	61	7.96
210	5.47	121	5.98	91	6.96
236	5.20	201	5.17	107	6.64
442	4.37	230	5.03	120	6.20
524	4.15	260	4.80	237	5.10
573	3.86	--------	2.98	264	4.88
				324	4.59
				578	3.78
				--------	3.39

THE CURVE EQUATION

After several trials, it was found that the equation

$$y = \frac{b}{x+a} - c,$$

where y = the water content, x = the tension, and a, b, and c are constants for the particular soil, seemed to give a satisfactory fit for the observations. The curve plotted by means of this equation is a hyperbola in which the value of y approaches the value of c as x increases beyond the limit of measurement. The value of c appears to represent an important characteristic of the soil, i. e., the unavailable water, which is the quantity of water that cannot be removed by any tension, however large. The equation was chosen empirically because it appeared to describe the conditions better than any other tried. It is not known to have any theoretical justification.

The method of evaluating the curve-equation constants may be illustrated by using the data from a calibration run made by the open-container method with a sample of composited surface soil, 0–8 inches, from a field in the South Farm at the [U. S.] Plant Industry Station, Beltsville, Md. For this soil, the record of the first run is

shown in table 10 and the data are plotted in figure 6.   In this experiment the tension scale was graduated in units of centimeters of water instead of millimeters of mercury.
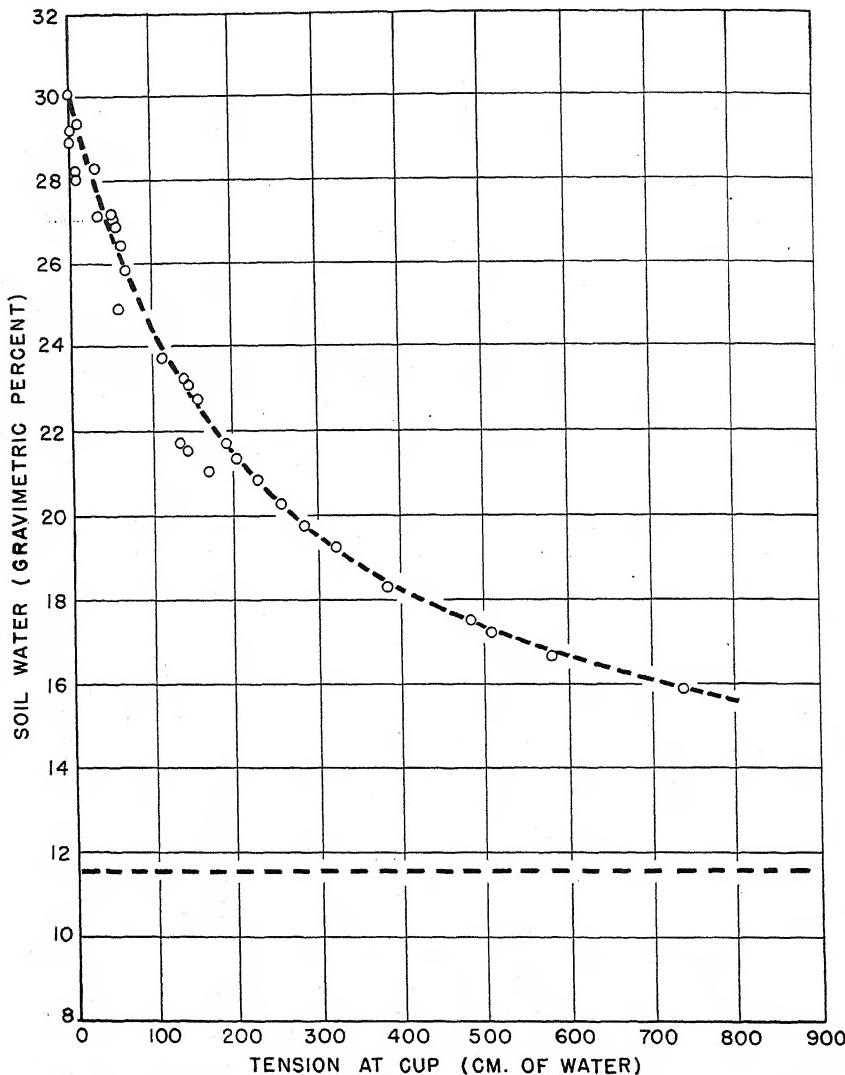
FIGURE 6.—The relation between tension (cm. water) and soil water, in Beltsville (Md.) silt loam, observed during the first run in an open container with orchard grass.   The line at the soil-water ordinate 11.6 is at the lower limit of the available soil water.

TABLE 10.—*Tension and soil-water values for Beltsville (Md.) silt loam, first run, July 10, 1944*

[Observations made between 8 a. m. and 4:45 p. m.]

Date	Clock time	Input	Daily loss	Tension	Soil water	
		Grams	Grams	Cm. H₂O	Grams	Percent
July 10	2:55	2,114		0	2,272	30.10
July 11	8:50			14	2,214	29.33
	1:00			3	2,204	29.20
	4:15			3	2,186	28.96
July 12	8:10		78	33	2,136	28.30
	1:00			10	2,129	28.21
	3:55			10	2,115	28.02
July 13	8:07		85	51	2,051	27.17
	12:45			50	2,048	27.13
	4:20			55	2,040	27.03
July 14	8:12		19	59	2,032	26.92
	4:45	43		34	2,049	27.15
July 15	8:15		79	65	1,996	26.44
	3:45	7		70	1,954	25.89
July 16	(¹)		123			
July 17	8:00		123	139	1,757	23.28
	10:35	213		143	1,748	23.16
	4:40			61	1,881	24.92
July 18	8:12		176	114	1,794	23.77
	4:32			154	1,715	22.72
July 19	8:15		155	190	1,639	21.71
	12:54			201	1,612	21.36
	4:15			227	1,573	20.84
July 20	8:05		111	254	1,528	20.24
	4:32			282	1,491	19.75
July 21	8:07		75	320	1,453	19.25
	4:22	331		382	1,383	18.32
July 22	8:15		143	131	1,641	21.74
	12:30			143	1,626	21.54
	3:30			169	1,586	21.01
July 23	(¹)		160			
July 24	8:09		160	482	1,321	17.50
	12:53			505	1,302	17.25
	4:21			578	1,259	16.68
July 25	8:13		118	734	1,203	15.94

¹ Sunday.

The three pairs of values chosen for use in the computation follow:

$$
\begin{array}{ccl}
x & y & \\
0 & 30.10 & (1) \\
201 & 21.36 & (2) \\
734 & 15.94 & (3)
\end{array}
$$

After the values for x and y have been selected, the values for a, b, and c may be obtained by the method of simultaneous equations. But it has been found that the essential computations may be made more expeditiously with a calculating machine as follows: [5]

Let:

$$
\begin{aligned}
x_1 y_1 &= \text{I} \\
x_2 y_2 &= \text{II} \\
x_3 y_3 &= \text{III} \\
x_1 - x_2 &= \text{IV} \\
x_2 - x_3 &= \text{V} \\
y_1 - y_2 &= \text{VI and} \\
y_2 - y_3 &= \text{VII}
\end{aligned}
$$

[5] The author is indebted to Francis Scofield for suggesting the use of the equation and for devising the method here shown for evaluating the constants.

Then:
$$c=\frac{(II-I)\ (VII)-(III-II)\ (VI)}{(IV)\ (VII)-(V)\ (VI)}=-11.62$$
$$a=\frac{II-I}{VI}-\frac{(IV)}{(VI)}\ c=224\ \text{and}$$
$$b=(y_1+c)\ (x_1+a)=4{,}140$$

From these computations it will be found that the value for c has a negative sign, whereas the values for a and b are positive. The curve equation for this run of this soil thus becomes:

$$\text{Total soil water}=\frac{4{,}140}{x+224}+11.62\ \text{or}$$
$$\text{Available soil water}=\frac{4{,}140}{x+224}$$

The curve yielded by the equation in the scale of total soil water is shown in figure 6, as is also the ordinate representing the $-c$ value, or the lower limit of the available soil water.

The points on the graph that fall below the curve represent observations that were made soon after the soil was watered on July 17 and again on July 21. These show that tensiometric equilibrium was not immediately attained after the water was added. The graph also shows, in the low-tension range, the effects on the observed tension of the diurnal changes of temperature in the soil. A rise in soil temperature when the soil is very wet may cause a decrease of tension. This phenomenon is less noticeable, or does not occur, when the soil becomes drier.

It is to be expected that, with successive runs on the same soil sample, changes in the water relations will occur as the soil becomes more compact and as the structure evolves. In the case of the Beltsville silt loam referred to above, the second run had its zero tension at 29 percent of water, whereas for the third and later runs it held 28 percent or slightly less at zero tension. For each of these later runs, the soil-water values when the upper limit of tension was reached were approximately the same as for the first run.

When, after several runs, the changes in the plotted-point path become inconsequential, it may be assumed that the relations then shown between tension and soil water are approximately the same as those existing in the field. At this time, a new set of x and y values may be chosen for the final evaluation of the curve-equation constants. The curve obtained with these values may then be superimposed on the plotted points for each of the runs to test its acceptability.

Because the values obtained for the constants depend upon the x and y values of the three points selected, it is well to use care in their selection. If data are available from several successive runs of the same soil or from runs with two or more containers of the same soil, the x and y values to be used in the computation may be obtained by the method of harmonic means. If data are available from only one run, such as with a soil core, the points may be evaluated by the method of least squares. But these latter calculations are tedious and may not be warranted. If the data are plotted carefully, it is not difficult to select three points that give a curve of acceptable fit for

any one run, or if there are several runs the harmonic means of points in the same range should be used.

The construction of a comparison curve and the testing of its fit to a series of observed points is facilitated by computing the data for a table to show the soil-water values for a series of tension values. Such a table for the Beltsville loam is here shown (table 11). In this table the values for available soil water and for total soil water are both given. If it is desired to test critically the fit of a computed curve to the data of a run, this may be done by using in such a table the observed tension values and then comparing the computed soil-water values with the observed values.

TABLE 11.—*Computed soil-water values for curve construction, Beltsville silt loam (composite), run of July 10, 1944*

Tension (cm. H_2O)	$x+a$	Available soil water $\left(\dfrac{b}{x+a}\right)$	Total soil water $\left(\dfrac{b}{x+a}-c\right)$	Tension (cm. H_2O)	$x+a$	Available soil water $\left(\dfrac{b}{x+a}\right)$	Total soil water $\left(\dfrac{b}{x+a}-c\right)$
		Percent	*Percent*			*Percent*	*Percent*
0	224	18.48	30.10	350	574	7.21	18.83
25	249	16.62	28.24	400	624	6.63	18.25
50	274	15.11	26.73	450	674	6.14	17.76
75	299	13.84	25.46	500	724	5.72	17.34
100	324	12.78	24.40	550	774	5.35	16.97
150	374	11.07	22.69	600	824	5.02	16.64
200	424	9.76	21.38	700	924	4.48	16.10
250	474	8.73	20.35	800	1,024	4.04	15.66
300	524	7.90	19.52				

CONVERSION TABLES

Upon the completion of the tensiometric calibration of the soil samples from a field location in which tensiometers have been installed, the next step is to set up a conversion table. It is quite possible to convert each reported tension value into its soil-water equivalent by direct computation, but in the long run the use of a conversion table saves time and diminishes the chances of error. It is no small task to construct a conversion table containing soil-water values for each of the 600 or 800 units of the tensiometer scale. For that reason, it is appropriate to give the subject careful advance consideration.

A conversion table may be made to show for each tension unit (1) the gravimetric values for total soil water, (2) the gravimetric values for available soil water, or (3) the volumetric values for available soil water. Which of these three should be used for any given situation is a question that merits serious consideration.

For use in connection with irrigation operations where the depth of water input may be measured and is usually reported in terms of feet, it has been found that the conversion table with the volumetric values for available soil water is the most convenient. Volumetric soil-water values may be computed from gravimetric soil-water values by multiplying the latter by the value for the apparent density of the soil. Thus, if for a certain tension the gravimetric soil-water value is 12 percent and the apparent density of that soil is 1.52, the volumetric soil-water value is 18.24 percent. Furthermore, a volumetric soil-water value of 18.24 percent is equivalent to 0.1824 foot of water in each foot of soil.

The operations involved in constructing a conversion table, which are at best laborious, may be lightened appreciably by substituting for the equation $y=\dfrac{b}{x+a}-c$, another equation, namely, $v=\dfrac{w}{ex+1}$, in which $w=$ the total available soil water, $e=$ the reciprocal of the curve-equation constant a, $x=$ the tension, and $v=$ the available soil water at the given tension. If it is desired to tabulate the values for total soil water, this may be done by adding to the value for v the value for the curve-equation constant $-c$.

EVALUATING THE WATER-HOLDING CHARACTERISTICS OF SOILS

The findings obtained from the tensiometric calibration of a sample of soil may serve as useful criteria for evaluating the water-holding characteristics of the soil. The calibration yields three important criteria: (1) The quantity of the total available soil water, for which the symbol w is used; (2) the quantity of the unavailable soil water, for which the symbol $-c$ is used; and (3) the factor e, which indicates the nature of the energy relations existing between the soil and its available water. The factor e is evaluated from $e=\dfrac{1}{a}$. Thus, the water-holding characteristics of the Beltsville silt loam herein reported on may be evaluated as follows:

$w=18.48$ percent (gravimetric)
$-c=11.62$ percent (gravimetric)
$e=\ \ .0045$, when the tension scale is in centimeters of water

If the tension scale were in millimeters of mercury, the value for e would be $0.0045\times1.35=0.0061$.

For the three soils used as examples in this report, the water-holding characteristics may be described concisely as follows:

Soil:	Available soil water (w) (percent)	Unavailable soil water (−c) (percent)	Value (e) (mm. Hg)
Rubidoux sandy loam, plot U	17. 30	4. 31	0. 0156
Lanham (Md.) sandy soil	15. 70	2. 70	. 0234
Beltsville (Md.) silt loam	18. 48	11. 62	. 0061

It will be seen, from the nature of the equation in which the factor e is used, that its value determines the shape of the curve. The relations are such that the higher its value the more pronounced is the bend of the curve.

SUMMARY AND CONCLUSIONS

To serve the purposes of agronomic investigations, it is convenient to recognize two categories of soil water, viz, available and unavailable. It is only the available soil water that is useful to plants or is free to move through the soil.

There are currently in general use three methods of measuring the quantity of water in the soil at any given time: (1) The gravimetric method, in which soil samples are weighed and dried to constant weight, and from the loss of weight the water content is computed; (2) an electrometric method, involving the measurement of the electrical resistance between two electrodes embedded in a block of gypsum placed in the soil; and (3) the tensiometric method, which

involves the measurement, with a mercury manometer, of the tension existing between the soil and its water.

In the first part of the investigation reported here, it was found that the tensiometer yielded precise and useful information on the status of the available soil water at any given horizon of the root zone throughout a range extending from the condition of saturation to that resulting from the withdrawal of 80 to 90 percent of the available soil water. The findings indicate that the instrument is highly sensitive to changes in the quantity of available soil water; that it gives consistent, dependable, and reproducible information; and that it is not greatly influenced by differences in soil or atmospheric temperatures or in the salinity of the soil solution. Thus, just as a thermometer may be used to show the temperature of the soil or of the air, so the tensiometer may be used to show the status of the available water in the soil.

This paper includes detailed reports of tensiometric observations at a number of field locations with a number of different crops. These observations show the changes in tension that occur from day to day at successive depths in the soil in response to, and following, inputs of water. They show also that the depth of the active root zone differs greatly between different crops grown on adjacent plots of the same soil.

In the second part of the investigation, it was found that it is possible to establish an acceptable relation between the data of tensiometric observations at any given field location and the quantity of available soil water at that location. This finding makes it possible to make up, for the soil of any given field location, a conversion table by the use of which the data of tensiometric field observations may be translated into (1) equivalent gravimetric percentages of available soil water, (2) equivalent volumetric percentages of available soil water, or (3) equivalent gravimetric or volumetric percentages of total soil water. Several methods of procedure to this end have been explored, and three of these methods are described.

The method of field sampling involves taking a number of soil samples from the area immediately adjacent to the tensiometer cup and comparing the tension reading with the mean value for total soil water as determined by drying the samples.

The method of soil cores in closed containers involves a series of successive changes in the quantity of water in the core with observations as to the tension after each change.

The method of open containers with growing plants involves a series of runs, for each of which the soil in the container is saturated, and the tension is observed and the weight of the system is recorded frequently as the plants absorb and transpire the soil water.

Of these three methods, the last-named yielded the most complete and acceptable assemblage of comparative data. When the findings from any one of the methods are plotted on coordinate paper, the path of the points forms a curve starting from zero tension, when the soil is saturated, and ending at some lower water percentage, when the upper limit of measurable tension is reached.

It has been found that the path of the points as plotted may be fitted with a curve computed by the use of the equation $y=\dfrac{b}{x+a}-c$, in

which $x=$ the tension value; $y=$ the soil-water value; and the constants a, b, and c are evaluated for each soil from three pairs of the values for tension and soil water. The value of $-c$ represents the quantity of unavailable water.

The evaluation of the curve-equation factors may also serve as a basis for a concise and precise description of the water-holding characteristics of the soil that has been used for tensiometric calibration. This in turn makes it possible to classify the soils of any location or the successive sections of the soil profile at any location, into categories that are delimited by their water-holding characteristics.

INHERITANCE OF RESISTANCE TO BUNT (TILLETIA CARIES) IN HYBRIDS WITH TURKEY WHEAT SELECTIONS C. I. 10015 AND 10016[1]

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INTRODUCTION

Studies of the inheritance of resistance in wheat to bunt (*Tilletia caries* (DC). Tul.) made at this station (*1, 2, 3, 4, 8*)[2] have demonstrated the existence of four major genetic factors for resistance to this disease. These factors, named for the variety in which they were discovered, are the Martin, Hussar, Turkey, and Rio factors. Briggs (*4*) has shown linkage between the Martin and Turkey factors with a crossover value of 34.22 percent. Stanford (*8*) found a close linkage between the Rio and Turkey genes with a recombination value of 2.4 percent.

As soon as a gene for resistance to bunt is discovered, it is used in test crosses with new varieties under study for the purpose of identifying their genes. When the present study was undertaken, Stanford (*8*) had not yet discovered the Rio gene; therefore the crosses with Rio were made at a later date and the data were obtained after Dr. El Khishen returned to Egypt. As will be seen later, this information has been very useful in the genetic analysis of Turkey 10016.

MATERIALS AND METHODS

The resistant parent varieties, Turkey C. I.[3] 10015 (Selection No. 4300) and Turkey C. I. 10016 (Selection No. 4319) are 2 of the 12 resistant lines isolated by Kiesselbach and Anderson (*5*) from Turkey (South Dakota 144) wheat. Other of their lines showed various amounts of bunt up to more than 90 percent. Turkey 10016 was found to have a higher level of resistance than Turkey 10015, and this has also been the case at the California station (table 1).

The two resistant varieties Turkey 10015 and Turkey 10016 were crossed with susceptible Baart, with the four tester varieties Martin (*MM*), Selection 1403 (*HH*), Turkey 3055 (*TT*), Rio (*RR*), and with each other.

The seeds for producing the F_1 and F_2 generations were treated with copper carbonate to prevent bunt infection which would lead to differential elimination of susceptible lines as well as some heterozygous plants in F_2. Since some genetically susceptible plants may escape infection, and some resistant plants may become diseased, the F_2 provides inadequate information; therefore the F_3 populations were used for the genetical analyses. Some rows of F_2 plants were grown with the F_3 to obtain an indication of the amount of bunt to be expected in F_3 rows arising from fully heterozygous F_2 plants.

All seeds used for the bunt nursery were thoroughly inoculated with bunt spores propagated on White Federation for that purpose.

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This collection of *Tilletia caries*, designated by Reed (*6*) as physiologic race III and by Rodenhiser and Holton (*7*) as T–1, has been used in all the smut studies at this station.

The seeds were spaced about 2 inches apart in rows 1 foot apart. The seeds were planted in November and harvested the following May and June. The number of plants per row averaged about 55. At harvest the plants were pulled and classified as smutted or disease-free. Plants which showed any of the disease were placed in the smutted class.

TABLE 1.—*Annual percentages of bunt infection in the parent varieties grown at Davis, Calif., 1933–43*

Year	Bunt infection (percent) on—						
	Baart	Turkey 10015	Turkey 10016	Martin	Selection 1403	Turkey 3055	Rio
1933	66.0	4.3	0	0	0	0.1	0
1934	84.4	4.5	.7	0	1.4	.3	.4
1935	82.2	8.8	0	0	1.7	0	1.1
1936	72.9	3.8	0	0	0	1.8	.2
1937	68.1	4.2	2.1	0	.9	1.0	.8
1938	54.0	.4	0	0	0	0	.2
1939	93.5	.7	.5	0	0	.7	.4
1940	89.7	3.6	.9	0	0	2.1	1.2
1941	45.4	0	0	0	0	0	0
1942	60.0	--------	--------	0	0	1.3	0
1943	77.7	3.9	.2	0	0	2.3	1.2
Average	72.2	3.4	.4	0	.4	.6	.5

EXPERIMENTAL RESULTS

Conditions were generally favorable for bunt development as shown by the level of infection in the Baart check rows and by the upper range of the homozygous susceptible rows. For convenience the data from hybrids involving Turkey 10015 and Turkey 10016 will be considered separately, although they appear in the same table.

INHERITANCE OF RESISTANCE TO BUNT IN HYBRIDS WITH TURKEY 10016

The F_3 data are given in table 2. Since these data are of importance principally as they relate to F_3, they will be discussed in connection with the F_3 data presented in table 3. There were 221 F_3 rows of Turkey 10016 $\times$ Baart with 2 Baart and 1 Turkey 10016 rows for about every 25 F_3 rows. The average infection of Turkey 10016 was 0.5 percent and for Baart 93.5 percent.

TABLE 2.—*Percentage of bunted plants in F_2 of 11 crosses at Davis, Calif.*

Year grown	Cross	Total plants	Bunted plants	
		Number	*Number*	*Percent*
1939	Turkey 10016 $\times$ Baart	1,734	356	20.4
1939	Turkey 10016 $\times$ Martin	2,277	45	1.9
1939	Turkey 10016 $\times$ Selection 1403	1,801	65	3.6
1939	Turkey 10016 $\times$ Turkey 3055	2,462	12	.5
1943	Turkey 10016 $\times$ Rio	660	1	.01
1939	Turkey 10015 $\times$ Baart	2,107	896	42.5
1939	Turkey 10015 $\times$ Martin	2,186	85	3.9
1939	Turkey 10015 $\times$ Selection 1403	1,949	187	9.6
1939	Turkey 10015 $\times$ Turkey 3055	2,378	59	2.5
1943	Turkey 10015 $\times$ Rio	150	11	7.3
1940	Turkey 10015 $\times$ Turkey 10016	592	13	2.2

TABLE 3.—*Distribution of F_8 rows of the crosses named into 5-percent classes for bunt infection; Davis, Calif.* [1]

Cross	Distribution of rows by 5-percent classes																					Total number of rows
	0	1–5	5–10	10–15	15–20	20–25	25–30	30–35	35–40	40–45	45–50	50–55	55–60	60–65	65–70	70–75	75–80	80–85	85–90	90–95	95–100	
Turkey 10016 × Baart	28	25	8	14	20	29	24	10	9	6	6	7	7	5	5	6	5	2	5	—	—	221
Turkey 10016 × Martin	145	54	24	13	5	3	4	0	0	1	0	1	—	—	—	—	—	—	—	—	—	250
Turkey 10016 × Turkey 3055	206	35	8	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	250
Turkey 10016 × Rio	218	31	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	240
Turkey 10016 × Selection 1403	106	52	25	13	14	9	9	6	4	4	0	1	2	0	1	0	1	0	1	—	—	248
Turkey 10015 × Baart	4	13	13	10	9	9	7	6	9	18	13	18	10	10	10	13	17	6	16	13	2	226
Turkey 10015 × Martin	93	56	43	22	9	6	10	2	2	1	1	3	—	—	—	—	—	—	—	—	—	249
Turkey 10015 × Turkey 3055	95	96	48	14	7	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	263
Turkey 10015 × Rio	37	79	23	35	14	8	6	3	—	—	—	—	—	—	—	—	—	—	—	—	—	234
Turkey 10015 × Selection 1403	77	36	23	29	12	10	11	5	8	11	2	4	2	2	4	3	1	3	1	2	—	246
Turkey 10015 × Turkey 10016	179	39	22	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	240

[1] The cross between Turkey 10015 and Turkey 10016 was grown in 1940. The crosses involving Rio were grown in 1943. All others were grown in 1939. The behavior of the parents and Baart during these years is shown in table 1.

Figure 1 shows the distribution of the F₃ rows on the basis of 5-percent classes. This curve might suggest a single Mendelian factor segregation if 7.5 and 45 percent are accepted as minima, thus dividing the population into 57 resistant, 116 segregating, and 48 susceptible rows. There was an average of 24.0 percent of bunt in segregating rows, which is in fair agreement with the 20.4 percent obtained for F₂. The curve depicted in figure 1 is not greatly different from some published by Briggs (1) for plants in which the segregation was that of a dominant resistant gene. The main difference is in the lack of a definite mode for the susceptible group in the cross under consideration here. The spreading effect in the susceptible group may be accounted for by a weak factor, as will be seen presently. There were no segre-
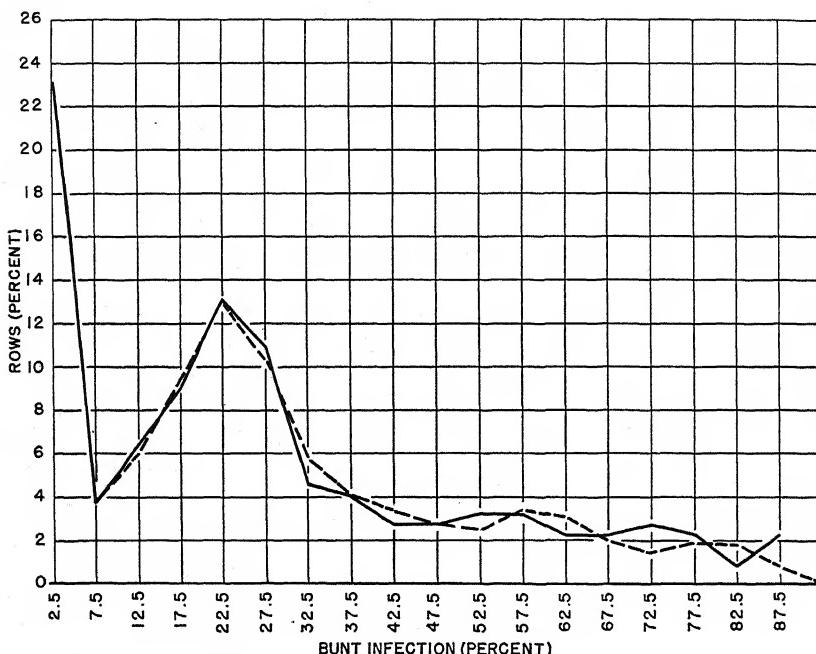
FIGURE 1.—Observed distribution of F₃ rows of Turkey 10016 × Baart for bunt infection (solid line) compared with the theoretical distribution (broken line).

gating or susceptible F₃ rows in the crosses of Turkey 10016 × Turkey 3055, which is the tester for the Turkey factor T, and with Rio, which is the tester for the Rio factor R. These data indicate that these two genes are present in Turkey 10016. The Rio factor was first reported by Stanford (8) in 1941. He showed that the Rio and Turkey genes were closely linked, with an estimated cross-over value of 2.4 percent. The data from Turkey 10016 × Rio were not available until 1943. Prior to that time a genetic analysis based on the Turkey factor and a weak factor pretty well satisfied the available data. Two closely linked genes will give a result similar to a single gene. Therefore it would have been impossible to detect the presence of two genes without the use of test crosses.

As pointed out earlier, a weak gene was postulated to account for the spread among susceptible rows. The addition of a weak factor is needed also to account for the 20.4 percent of bunt in F_2. The percent of bunt to be expected in F_2 rows from the T and R genes entering the cross in the coupling phase is not known exactly, but it would be considerably above 20 percent. It is not known how much protection heterozygous T and R ($TtRr$) gives plants, but it is known that about half the plants heterozygous for T or R become infected. These, together with any infected plants from the $TtRr$ group, plus the 22.7 percent of susceptible plants expected, would probably amount to as much as 35 percent of bunted plants. As will be seen later, the weak factor postulated here is found also in Turkey 10015. Therefore there seems little doubt about its presence. For convenience, this gene will be designated as X.

The information available on the X factor indicates that it allows about 25 percent of bunted plants when homozygous and about 75 percent when heterozygous. The T and R genes give almost complete protection when homozygous but permit about half the heterozygous plants to become infected. The Rio gene is a little weaker in this respect than the Turkey. As pointed out above, the interaction of genes cannot be calculated accurately as would be the case if only susceptible plants become infected. The values used are consistent with what might reasonably be expected. The final decision as to the exact value used was dictated by the requirements of the distribution observed in figure 1. With the exception of the triple heterozygote, the various genotypes permitting bunt were assigned mean values which were multiples of 2.5 percent. Further refinement did not seem to be justified since it would furnish little additional useful information. In the case of the triple heterozygote, 24 percent was used for the mean because it resulted in a considerably better fit than the 20.4 percent which was observed in F_2.

The proportion of F_2 genotypes, the number of F_3 rows expected from each out of a total of 221 rows, and the percent of bunt expected in F_3 rows are given in table 4. Stanford's cross-over value of 2.4 percent was used for the T–R relationship. X is independent.

No attempt was made to assign values to the F_3 rows marked resistant, although it is recognized that these would differ slightly in resistance. Frequency distributions were calculated for the other genotypes by using the means indicated and standard errors derived from the formula $S = \sqrt{\dfrac{pq}{n}}$. While this probably underestimates the errors since no account is taken of environmental effects, it does give a distribution which conforms very well to that observed. The separate distributions for the genotypes concerned are shown in table 5.

The combined values of the separate distributions give the number of rows expected for the various classes. The 63.31 resistant rows expected were arbitrarily assigned to the first three classes, using the available rows in the first two classes and placing the remaining in the third class. This resulted in complete agreement between expected and observed rows in the first two classes. However, the agreement between observed and expected beyond this class is very good, as will be seen in figure 1. The χ^2 for classes beyond those to which rows were assigned arbitrarily is 3.5009, which gives a very high probability.

TABLE 4.—*Proportion of F_2 genotypes expected, number of F_3 rows expected out of a total of 221, and percent of bunt expected in F_3 rows of Turkey 10016 × Baart*

Proportion of F_2 genotype expected	Number of F_3 rows expected	Mean percent of bunt expected in F_3 rows	Proportion of F_2 genotype expected	Number of F_3 rows expected	Mean percent of bunt expected in F_3 rows
$0.0566\ \frac{TR\ X}{TR\ X}$	12.52	(1)	$0.00015\ \frac{Tr\ X}{Tr\ X}$	0.03	(1)
$.1133\ \frac{TR\ X}{TR\ x}$	25.04	(1)	$.0003\ \frac{Tr\ X}{Tr\ x}$	.07	(1)
$.0567\ \frac{TR\ x}{TR\ x}$	12.52	(1)	$.00015\ \frac{Tr\ x}{Tr\ x}$	.03	(1)
$.0057\ \frac{TR\ X}{Tr\ X}$	1.26	(1)	$.0057\ \frac{Tr\ X}{tr\ X}$	1.26	(1)
$.0114\ \frac{TR\ X}{Tr\ x}$	2.52	(1)	$.0114\ \frac{Tr\ X}{tr\ x}$	2.52	25
$.0057\ \frac{TR\ x}{Tr\ x}$	1.26	(1)	$.0057\ \frac{Tr\ x}{tr\ x}$	1.26	40
$.0057\ \frac{TR\ X}{tR\ X}$	1.26	(1)	$.00015\ \frac{tR\ X}{tR\ X}$	.03	(1)
$.0114\ \frac{TR\ X}{tR\ x}$	2.52	(1)	$.0003\ \frac{tR\ X}{tR\ x}$	.07	(1)
$.0057\ \frac{TR\ x}{tR\ x}$	1.26	(1)	$.00015\ \frac{tR\ x}{tR\ x}$	.03	(1)
$.1133\ \frac{TR\ X}{tr\ X}$	25.04	17.5	$.0057\ \frac{tR\ X}{tr\ X}$	1.26	(1)
$.2266\ \frac{TR\ X}{tr\ x}$	50.10	24	$.0114\ \frac{tR\ X}{tr\ x}$	2.52	25
$.1133\ \frac{TR\ x}{tr\ x}$	25.04	40	$.0057\ \frac{tR\ x}{tr\ x}$	1.26	50
$.0003\ \frac{Tr\ X}{tR\ X}$	.07	(1)	$.0567\ \frac{tr\ X}{tr\ X}$	12.52	25
$.0006\ \frac{Tr\ X}{tR\ x}$	.13	(1)	$.1133\ \frac{tr\ X}{tr\ x}$	25.04	60
$.0003\ \frac{Tr\ x}{tR\ x}$	.07	(1)	$.0567\ \frac{tr\ x}{tr\ x}$	12.52	80

[1] Resistant.

Segregation occurred in the crosses of Turkey 10016 with Selection 1403, which carries the Hussar factor H, and with Martin, which carries the M factor for resistance to bunt. While no extensive analysis has been made of the segregation in these crosses, the number of susceptible and segregating rows are in general conformity with the hypotheses advanced above. The lack of susceptible rows in the crosses with Martin is the result of linkage between the Martin, Turkey, and Rio factors.

The available data clearly show that Turkey 10016 carries the Turkey and the Rio factors for resistance to bunt. An additional weak factor which has been designated X is necessary to explain adequately the segregation observed. The data indicate that this factor allows about 25 percent of diseased plants when in the homozygous condition. While such a weak factor has not been previously reported, varieties of wheat with this type of resistance are known to occur. Mackie and Briggs in 1920 (unpublished data) found 6 out of 956 varieties and strains of wheat which showed a bunt infection between 20 and 25 percent. This same weak factor must be considered in explaining the segregation observed in the cross between Turkey 10015 and Baart.

TABLE 5.—*Theoretical distributions of F₃ rows of Turkey 10016 x Baart for the several genotypes indicated*

[Numbers of rows and means used for genotypes are those shown in table 4]

Class centers	$\frac{TR\ X}{tr\ X}$	$\frac{TR\ X}{tr\ x}$	$\frac{TR\ x}{tr\ x}$	$\frac{Trx}{tr\ x}$	$\frac{Trx}{tr\ x}$	$\frac{tR\ X}{tr\ x}$	$\frac{tRx}{tr\ x}$	$\frac{tr\ X}{tr\ X}$	$\frac{tr\ X}{tr\ x}$	$\frac{tr\ x}{tr\ x}$	Resistant rows	Expected rows	Observed rows	x^2/m [1]
											Number	*Number*	*Number*	
2.5	0.19										52.81	53.00	53	
7.5	1.62										6.38	8.00	8	
12.5	6.04	2.84		0.11		0.11		0.52			4.12	13.74	14	
17.5	9.34	9.23		.38		.38		1.90				21.23	20	0.0713
22.5	6.04	16.44	0.27	.77		.77		3.85				28.14	29	.0263
27.5	1.62	14.25	1.30	.77	0.06	.77		3.85				22.62	24	.0842
32.5	.19	5.97	3.98	.38	.22	.38	0.02	1.90				13.04	10	.7087
37.5		1.33	6.97	.11	.36	.11	.07	.52				9.47	9	.0233
42.5			6.97		.36		.20					7.53	6	.3109
47.5			3.98		.22		.34		1.57			6.11	6	.0020
52.5			1.30		.06		.34		3.98			5.68	7	.3068
57.5			.27				.20		6.97			7.44	7	.0260
62.5							.07		6.97			7.04	5	
67.5							.02		3.98	0.38		4.38	5	.1766
72.5									1.57	1.80		3.37	6	
77.5										4.09		4.09	5	1.6798
82.5										4.09		4.09	2	
87.5										1.80		1.80	5	.0850
92.5										.38		.38		
Total	25.04	50.06	25.04	2.52	1.28	2.52	1.26	12.54	25.04	12.54	63.31	221, 15	221	3.5009

[1] Square of difference between number observed and number expected, divided by number expected.

INHERITANCE OF RESISTANCE TO BUNT IN HYBRIDS WITH TURKEY 10015

As will be seen in table 1, Turkey 10015 is highly resistant to bunt, although it usually shows a little more of this disease than Turkey 10016.

The F₃ data from crosses of Turkey 10015 with Baart, with the four test varieties and with Turkey 10016, are given in table 3. The segregation with Baart is shown in figure 2. The small proportion of
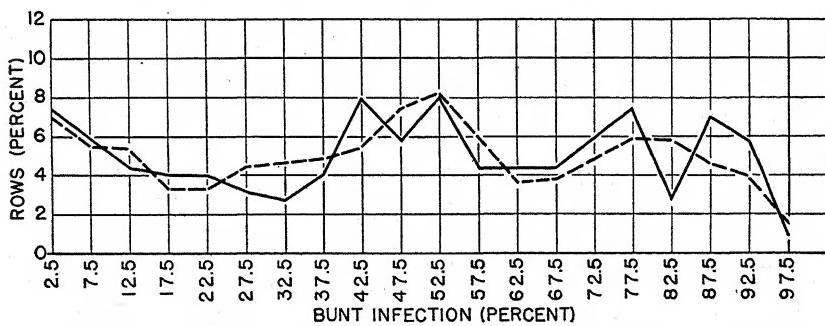
FIGURE 2.—Observed distribution of F₃ rows of Turkey 10015×Baart for bunt infection (solid line) compared with the theoretical distribution (broken line).

highly resistant rows, together with the absence of prominent modes, suggests the lack of a strong factor. Segregation occurred in the crosses with Martin, Turkey 3055, Selection 1403, and Rio, showing definitely that none of the four factors carried by these varieties is present in Turkey 10015. No segregation occurred, however, in the cross with Turkey 10016; these two must, therefore, have the X resistant factor in common.

An attempt has been made to satisfy the segregation encountered in the cross with Baart with an analysis which would be consistent with known facts. At best, the interpretation probably only approximates the true condition and therefore the information will have limited usefulness.

Several theories were followed up, assuming the presence of one or two weak factors in addition to the X factor which is known to be present. While three weak factors generally gave a slightly better fit than two, the reduction in χ^2 did not seem to be sufficient to justify this theory; therefore the analysis presented is based on two weak factors. The approach was the same as that used for Turkey 10016 ×Baart.

As pointed out above, the standard errors used probably are too small. Again, it should be emphasized that the interaction of factors cannot be predicted accurately from the action of the factors entering into the combination. The F_2 genotypes and the mean percentages of bunt assumed for F_3 rows are given below.

Ratio of F_2 genotype:	Mean percent of bunt in F_3 rows	Ratio of F_2 genotype—Con.	Mean percent of bunt in F_3 rows
1 $XXYY$	2.5	2 $Xxyy$	72.5
2 $XXYy$	10.0	1 $xxYY$	45.0
2 $XxYY$	32.5	2 $xxYy$	82.5
4 $XxYy$	52.5	1 $xxyy$	92.5
1 $XXyy$	22.5		

The values used for XX and Xx were 22.5 and 72.5, as compared with 25.0 and 60.0 assigned them on the basis of the data from Turkey 10016×Baart. Since these values are only approximate, the use of slightly different values seems justified. It was assumed that YY would permit 45.0 percent of diseased plants and Yy 82.5 percent in F_3 rows.

The F_2 data (table 2) indicated that F_3 rows arising from $XxYy$ plants should have an average of 42.5 percent of bunt. When such a value was used it was necessary to assign YY a very weak value. The interaction of such a weak gene with XX would hardly be expected to confer so high a degree of resistance on $XXYY$ individuals as that observed. Therefore, a value of 52.5 percent was assigned to the double heterozygote. The values assigned to the other genotypes are consistent with those reasonably expected.

Table 6 shows the distributions for the various genotypes which were calculated for the 226 F_3 rows of Turkey 10015×Baart, using the means indicated above. The agreement between the rows observed and those expected is satisfactory; χ^2 is 18.2597, which gives a probability greater than 0.3.

The segregation observed in the cross between Turkey 10015 and Selection 1403 confirms the above hypothesis, insofar as an analysis has been attempted. On the basis of 3 factors 3.8 susceptible rows would be expected. The exact number present cannot be stated definitely, but it may be assumed that 3 or 4 of the rows present are homozygous for susceptibility. As shown by the distributions in table 6, 60 percent of bunt would roughly divide genotypes $hhXxyy$, $hhxxYy$, and $hhxxyy$ from the others. Thus, $\frac{5}{64}$ or 19.2 rows would be expected with percentages of bunt above this point. Fourteen such rows were observed. In the cross of Turkey 10015 with Martin there was 1 row above 60 percent, but in the crosses with Turkey 3055 and

Rio there was none. This deficiency of rows would result from linkage. It is known that the Martin, Turkey, and Rio genes are all linked; therefore all these crosses should show such a deficiency if the Y factor is linked with them. With a cross-over of 15 percent, which would put the Y gene about an equal distance from the 3 other genes, 4 or 5 rows with bunt above 60 percent would be expected. This is more than was actually observed.

TABLE 6.—*Theoretical distribution of F_3 rows of Turkey 10015 × Baart for the several genotypes indicated*

[Means used for genotypes are those shown in tabulation, p. —]

Classes	$XXYY$	$XXYy$	$XxYY$	$XxYy$	$XXyy$	$Xxyy$	$xxYY$	$xxYy$	$xxyy$	Number of rows expected	Number of rows observed	x^2/m [1]
0–5	12.80	3.10								15.90	17	0.0761
5–10	1.30	11.02			0.20					12.52	13	.0184
10–15		11.02			1.12					12.14	10	.3772
15–20		3.10	0.70		3.34					7.14	9	.4845
20–25			2.67		4.82					7.49	9	.3044
25–30			6.45		3.34		0.19			9.98	7	.8898
30–35			8.64		1.12		.79			10.55	6	1.9623
35–40			6.45	1.84	.20		2.27			10.76	9	.2879
40–45			2.67	5.76			3.82			12.25	18	2.7084
45–50			.70	12.52			3.82			17.04	13	.9578
50–55				16.24			2.27			18.51	18	.0141
55–60				12.52		0.55	.79			13.86	10	1.0750
60–65				5.76		2.50	.19			8.45	10	.2843
65–70				1.84		6.57		0.22		8.63	10	.2175
70–75						9.04		1.85		10.89	13	.4088
75–80						6.57		6.81		13.38	17	.9794
80–85						2.50		10.52	0.25	13.27	6	3.9829
85–90						.55		6.81	3.17	10.53	16	2.8415
90–95								1.85	7.28	9.13	13	} .3894
95–100						.22			3.42	3.64	2	
Total	14.10	28.24	28.24	56.48	14.14	28.28	14.14	28.28	14.12	225.78	226	18.2597

[1] Square of difference between number observed and number excepted, divided by number expected.

Obviously, the final test of the above theory will come when selections from Turkey 10015 × Baart are available, each of which is homozygous for the X or Y factor only.

DISCUSSION AND SUMMARY

A genetic analysis of Turkey 10016 revealed the presence of one weak and two strong factors for resistance to bunt (*Tilletia caries*). The two strong factors were identified as the Turkey factors, previously reported by Briggs (*3*), and the Rio factor, reported by Stanford (*8*). These had been shown by Stanford (*8*) to be linked, with an estimated cross-over of 2.4 percent. Entering the cross in the coupling phase, the segregation was quite similar to that expected from a single strong factor. The complete analysis, therefore, would not have been possible if Turkey 3055 and Rio had not been available to test for the presence of their genes.

A third gene pair designated as XX was necessary to explain the type of segregation observed among the more susceptible F_3 rows. This gene was different from any previously reported from this laboratory in that it permitted about 25 percent of bunt when homozygous for resistance. A few varieties are known which permit a like

amount of bunt under similar conditions. The presence of the X factor is necessary, furthermore, to explain the lack of segregation in the cross with Turkey 10015.

Turkey 10016 should be a valuable parent for the plant breeder because of the presence of two strong genes for resistance to bunt which are closely linked. Almost 80 percent of the rows homozygous for resistance in F_3 will be homozygous for both pairs of resistant genes.

The genetic analysis of Turkey 10015 left much to be desired. As indicated above, the X gene must be present. The presence of an additional weak gene Y which permits 45.0 percent of bunt when homozygous for resistance will satisfy the segregation observed in F_3 of Turkey 10015 $\times$ Baart. It should be emphasized that other gene combinations and values might satisfy the observed distribution. The one chosen is based on the minimum number of genes. Furthermore, the values assigned to the various genotypes are in accordance with reasonable expectations based on each of the two gene pairs acting alone.

There are some indication that the Y gene is linked with the Martin, Turkey, and Rio genes which are known to be linked with each other ($4, 8$). While it does not seem desirable to stress the probable linkage of the Y factor until conclusive evidence is available, it is of interest that three of the four strong genes and probably one of the two weak genes for bunt resistance thus far discovered are carried by a single chromosome pair. Common wheat is a hexaploid species and one in which duplicate and triplicate factors have been reported on several occasions. Since almost no linkage has been reported, this evidence has been used to support the allopolyploid origin of wheat, assuming that similar genes affecting the same character have been contributed by different parent species. No question is raised here regarding the origin of wheat. Multiple factors are known to exist in diploid species. Seven genes for resistance to mildew in barley, which is a diploid species, have been reported from this laboratory (9). It would appear that most of the genes for resistance to bunt thus far discovered have been derived from one of the parent species. There is a possibility that these resistant genes have piled up on this chromosome pair as a result of the occasional crossing over between nonhomologus chromosomes or between similar chromosomes of different genomes which is believed to occur in wheat. It does not seem likely that genes for resistance arose by duplication of the same gene since they differ in their reaction to some races of bunt.

The fact that two weak genes acting together confer such a high degree of resistance as that observed in Turkey 10015 is of importance to the plant breeder. Although such genes probably would not be used where strong ones are available, combinations of weak genes might be very useful in breeding for disease resistance in cases where no strong genes are available.

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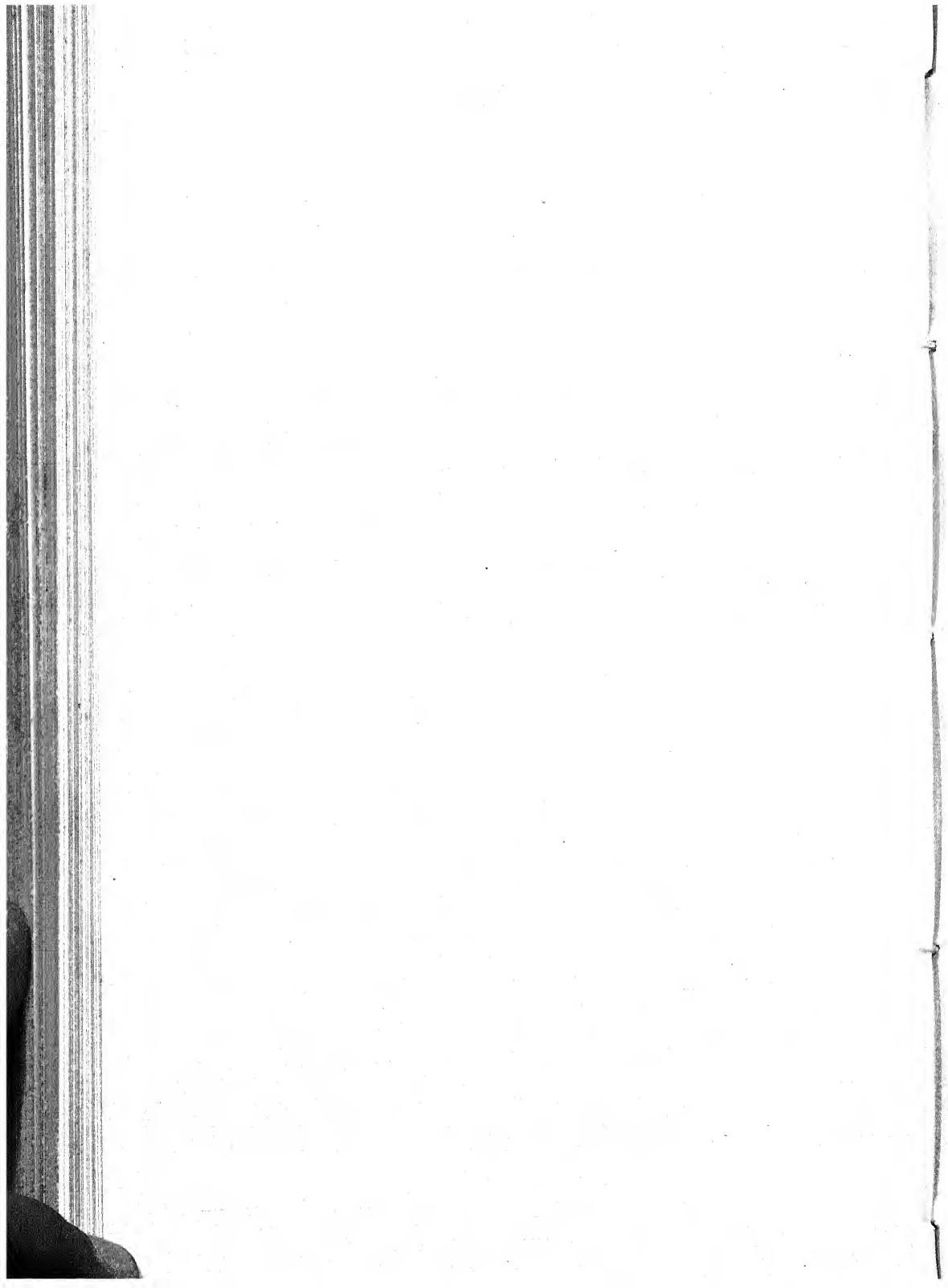
THE ACTION OF A REPELLENT SPRAY AGAINST THE MEXICAN FRUITFLY [1]

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INTRODUCTION

When studies on repellents for the Mexican fruitfly (*Anastrepha ludens* (Loew)) were begun in Morelos, Mexico, in 1939, it was assumed that an effective repellent would reduce the fruitfly population, and that the reduction could be measured by comparing the numbers of fruitflies caught in traps. During the course of experiments in an orange grove in 1940, however, it was noted that the traps in sprayed trees sometimes yielded more flies than traps in comparable unsprayed trees, although counts on the infested oranges indicated that the sprays may have provided some protection.

This apparent contradiction led to the hypothesis that, although for a short time after the application a reduction in population is accompanied by a reduced catch, there is later an increased catch, owing possibly to the unusual activity of the flies in flitting from one repellent surface to another as the odor of the repellent becomes less pronounced. In other words, a negative phase in the trap catches is followed by a positive phase, each of which may misrepresent the true fruitfly population. The testing of this hypothesis was made the objective of studies in 1941.

METHODS AND MATERIALS

In the experiments in 1940 counts were made every 3 or 4 days, which was long enough after spraying to pass the negative phase completely. Therefore, in testing the hypothesis, counts might be needed two or three times daily, as well as a sufficient number of flies per sample to give a reliable index of the population, and adequate replication for testing the significance of differences. Since the fruitfly population in an orange grove was generally too small to give adequate catches in less than 2 days, mango trees, in which flies are more abundant, were used in these tests. The tests were made in Cuernavaca, in the State of Morelos.

Two trees 14 feet apart were selected; one was sprayed about once a week with the repellent, the other was left unsprayed as a control. Five traps were placed in representative positions in each tree. They were removed during each spray application and replaced when the leaves were dry. Since it was necessary to use knapsack spray equipment and to climb the tree, coverage at the top, which was 30 feet high, was not so good as might have been desired.

The spray mixture was prepared from commercial materials by mixing 135 ml. of a medium-weight mineral oil, 13.5 ml. of oleic acid, and 27 ml. each of amyl acetate, cyclohexylamine, dicyclohexylamine,

and 99-percent *l*-nicotine. The last four compounds had been found to be repellent to *Anastrepha ludens* in previous experiments. The mineral oil was included as a fixative, and the oleic acid to form a soap emulsifier with the organic bases. The nicotine was isolated from a commercial nicotine sulfate solution (50 percent nicotine) and used without distillation. This mixture was poured into 9 liters of tap water. An emulsion was formed readily, and the entire amount was sprayed on the test tree.

The glass traps contained 200 ml. of a lure consisting of an 8-percent sugar solution fermented by 0.3 gm. of dried beer yeast. The traps were set in the trees on March 25, and the first spray was applied on March 27. Subsequent sprays were applied about once a week until June 30, when the fruit was picked.

TRAP DATA

Counts of *Anastrepha ludens* were recorded usually one-half hour before each spraying and at various intervals after spraying.

To measure the effect of the repellent spray, the total count in five traps from the test tree was compared with a similar total from the control tree. The ratio was used, but to give a reduction in catch the same range as the increase in catch, the ratio was converted to its logarithm. The negative logarithms represented a reduction and the positive logarithms an increase in catch. The trap data, together with the ratios and their logarithms, are given in table 1.

TABLE 1.—*Comparison of trap catches of Anastrepha ludens in a sprayed and an unsprayed mango tree, 1941*

Spray application	Date	Time after spraying [1]	Fruitflies caught in 5 traps		Ratio	Log *R*
			Sprayed tree	Control tree		
	Mar. 27:	*Hours*	*Number*	*Number*		
	7:30 a. m.	−½	65	85	0.76	−0.1
1	1 p. m.	5	10	20	.50	−.30
	6 p. m.	10	28	21	1.33	.12
	Mar. 28:					
	1 p. m.	29	15	18	.83	−.08
	Mar. 31:					
	7:30 a. m.	−½	87	117	.74	−.13
2	12 m.	4	17	22	.77	−.11
	6 p. m.	10	33	16	2.06	.31
	Apr. 1:					
	1 p. m.	29	31	13	2.38	.38
	Apr. 3:					
	7:30 a. m.	−½	102	76	1.34	.13
	12 m.	4	9	16	.56	−.25
3	6 p. m.	10	31	33	.94	−.03
	Apr. 4:					
	1 p. m.	29	20	11	1.82	.26
	Apr. 5	50	51	34	1.50	.18
	Apr. 7		184	133	1.38	.14
	Apr. 11		225	283	.80	−.10
	Apr. 15:					
	7:30 a. m.	−½	372	347	1.07	.03
4	11 a. m.	3	12	50	.24	−.62
	6 p. m.	10	173	196	.88	−.05
	Apr. 16:					
	11 a. m.	27	99	79	1.25	.10
	Apr. 18		422	518	.81	−.09
	Apr. 21:					
	7:30 a. m.	−½	485	570	.85	−.07
5	11 a. m.	3	55	47	1.17	.07
	6 p. m.	10	221	207	1.07	.03
	Apr. 22:					
	12 m.	28	192	137	1.40	.15
	Apr. 25		901	962	.94	−.03

See footnote at end of table.

TABLE 1.—*Comparison of trap catches of Anastrepha ludens in a sprayed and an unsprayed mango tree, 1941*—Continued

Spray application	Date	Time after spray-ing [1]	Fruitflies caught in 5 traps		Ratio	Log R
			Sprayed tree	Control tree		
		Hours	*Number*	*Number*		
	Apr. 29:					
	7:30 a. m.	$-\frac{1}{2}$	992	942	1.05	.02
6	11 a. m.	3	63	79	.80	−.10
	6 p. m.	10	108	153	.71	−.15
	Apr. 30:					
	11 a. m.	27	147	152	.97	−.01
	May 2		588	587	1.00	.00
	May 6:					
	7:30 a. m.	$-\frac{1}{2}$	718	709	1.01	.01
7	11 a. m.	3	16	29	.55	−.26
	6 p. m.	10	106	86	1.23	.09
	May 7:					
	11 a. m.	27	55	57	.96	−.02
	May 9		608	679	.90	−.05
	May 12:					
	7:30 a. m.	$-\frac{1}{2}$	379	324	1.17	.07
	12 m.	4	18	25	.72	−.14
8	7 p. m.	11	79	26	3.04	.48
	May 13:					
	11 a. m.	27	32	22	1.45	.16
	5:30 p. m.	$33\frac{1}{2}$	74	84	.88	−.05
	May 15		165	205	.80	−.09
	May 19:					
	8 a. m.	−1	226	325	.70	−.16
9	12 m.	3	3	2	1.50	.18
	6 p. m.	9	18	23	.78	−.11
	May 20:					
	12 m.	27	36	54	.67	−.18
	May 23		385	372	1.03	.01

[1] A minus sign indicates the time before spraying. Each spray was applied at 8 a. m. except No. 9, which was applied at 9 a. m. Where no figure is given, the exact time of examination was not recorded.

EFFECT OF REPELLENT SPRAY

The effect of the repellent spray on the trap catch is illustrated by the histogram in figure 1. The mean value (−0.024) of the log R values for the catch before the application of spray was used as the base of the histogram rather than zero. The mean log R value for counts made 3 to 5 hours after spraying was −0.170, for counts after 9 to 11 hours 0.076, after 27 to 29 hours 0.084, and after 3 to 4 days −0.012. The shaded rectangles therefore indicate approximately the negative, positive, and equilibrium phases of the catch.

The log R values were examined statistically by the method of the analysis of variance. The significance of the variance between results for certain time intervals was first determined. These values for 3 to 5 hours after spraying were taken as a measure of the negative phase of the catch. The values obtained from the count at 9 to 11 hours were omitted, except as explained later, because they usually fell between the depth of the negative phase and the peak of the positive phase, and in replicate trials the catch at this time would be positive or negative depending on whether the field conditions increased or decreased the evaporation of the repellent. Inclusion of this count would be expected to give an unfavorable bias to the experimental error, since no effort was made to control the evaporation of the repellent, but only the relative catch of *Anastrepha ludens*.

The appropriate measure of the positive phase of the catch under field conditions could not be expected at the same time after spraying
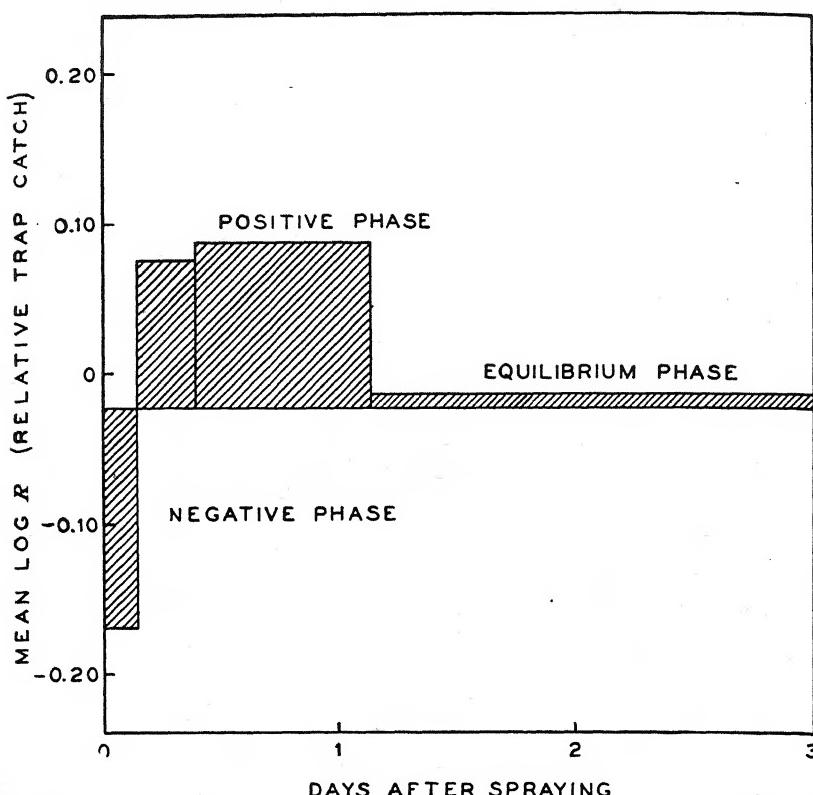
FIGURE 1.—The effect of repellent spray on trap catch of *Anastrepha ludens.*

in all applications. For example, following applications at relatively high field temperatures, wind velocities, and evaporation rates, the trap catch would pass through the negative phase and into the positive faster than after other applications. A continuation of those conditions would lead to the evaporation of the small amount of repellent substance required to maintain the positive phase and hasten the return to equilibrium. Therefore, an attempt to take the measure of the positive phase at any fixed time after spraying, such as 27 to 29 hours, would result in its underestimation. The desired arrangement would be to follow the curve continuously along with appropriate weather records, but counts must be large enough to give a reliable ratio and often several hours are required. In the analysis of the data the peak ratio of the positive phase has been used, provided a negative phase immediately after spraying was followed by a positive phase.

The analysis of variance was used to test the significance of the negative and positive phases, but not their significance at any given time, although it was relatively easy to fix the measure of the negative phase at 3 to 5 hours. The positive phase was measured after six applications at 27 to 29 hours and after three applications at 9 to 11 hours. Since the first three applications were made only 4 and 3 days apart, the equilibrium value for each of the first two applications is the same as the before-spraying value for the succeeding application. The differences between the before-spraying and equilibrium values

were generally so small as to make very little change in the variance. Toward the end of the experiment there was noticeable protection of the sprayed fruit.   Therefore, spraying was continued to save the fruit rather than to extend the trap data, and counts were taken after 3 or 4 hours as a routine check on the repellent action.   In three out of four applications the reduction in catch was 40 percent or more. The data as analyzed are given in table 2.

TABLE 2.—*Comparative trap catch of Anastrepha ludens, in a sprayed and an unsprayed tree, expressed as log ratios, before spraying and in different phases after spraying*

Spray application	Trap catch of *A. ludens*—				
	Before spraying	After spraying			Total
		Negative phase	Positive phase	Equilibrium phase	
1	−0.12	−0.30	[1] 0.12	−0.13	−0.43
2	−.13	−.11	.38	.13	.27
3	.13	−.25	.26	.14	.28
4	.03	−.62	.10	−.09	−.58
5	−.07	.07	.15	−.03	.12
6	.02	−.10	−.01	.00	−.09
7	.01	−.26	[1] .09	−.05	−.21
8	.07	−.14	[1] .48	−.09	.32
9	−.16	.18	−.18	.01	−.15
Total	−0.22	−1.53	1.39	−0.11	−0.47
Mean [2]	−.024	−.170	.154	−.012	

ANALYSIS OF VARIANCE

Source of variation	Degrees of freedom	Sum of squares	Variance
Application	8	0.2099	0.02624
Phase	3	.4754	[3] .15847
Error	24	.6801	.02834
Total	35	1.3654	

[1] 9- 10-hour values.
[2] Minimum significant difference between means, 0.164.
[3] Highly significant.

The analysis of variance measures the degree of significance of the differences indicated graphically in figure 1.   The repellent spray produced a highly significant shift in the comparative trap catches of *Anastrepha ludens* in the two trees.   The mean for the positive phase was significantly different from that before spraying and that for the equilibrium phase.   The experimental data therefore strongly indicate the existence of the negative phase followed by a positive phase in the action of a repellent on the trap catch of *A. ludens* in a mango tree.

EFFECT OF TIME OF DAY

As a check on the conclusions drawn from the foregoing analysis, the ratio of the trap data in the two trees was studied without the presence of repellent spray.   McPhail [2] has shown how the trap catch varies during the day, with relatively small catches early in the morning and late in the afternoon.   Presumably these differences would occur in both test and control trees, and the ratio would remain constant during the entire day.

[2] McPhail, M.   RELATION OF TIME OF DAY, TEMPERATURE AND EVAPORATION TO ATTRACTIVENESS OF FERMENTING SUGAR SOLUTION TO MEXICAN FRUITFLY.   Jour. Econ. Ent. 30: 793–799, illus.   1937.

The uniformity trials were run during the spray program when the ratio had returned to equilibrium after a spray application. Counts were taken late in the afternoon, early in the morning, and in the late forenoon. The data are presented in table 3.

TABLE 3.—*Comparison of the trap catch of Anastrepha ludens in the 2 mango trees without the presence of repellent spray, 1941*

Uniformity trial	Date	Fruitflies caught in 5 traps		Ratio	Log R
		Test tree	Control tree		
	Apr. 22:	*Number*	*Number*		
	6:30 p. m.	235	220	1.07	0.03
1	Apr. 23:				
	7:30 a. m.	6	20	.30	−.52
	11:30 a. m.	146	112	1.30	.12
	6 p. m.	158	163	.97	−.01
2	Apr. 24:				
	7:30 a. m.	21	55	.38	−.42
	11:30 a. m.	147	159	.92	−.03
	6 p. m.	111	175	.63	−.20
3	Apr. 25:				
	7:30 a. m.	1	6	.17	−.78
	11:30 a. m.	76	52	1.46	.16
	Apr. 30:				
	6 p. m.	234	241	.97	−.01
4	May 1:				
	7:30 a. m.	23	33	.70	−.16
	11:30 a. m.	96	111	.86	−.06

From an inspection of the data it seems likely that the first flies caught in the morning are in the control tree, and that any experiment based on catches between 6 p. m. and 7:30 the following morning will be biased in favor of a larger catch in the control tree.

In the repellent-spray experiment the catches from 6 p. m. to 7:30 a. m. were combined for analysis with either the forenoon or the afternoon catches. The appropriate uniformity trials for the experiment would therefore consider the night counts combined with one of the other counts. Since combining with afternoon counts introduces the greater variation, the data were analyzed in that manner. The combined data in terms of log R and a summary of the analysis are presented in table 4.

TABLE 4.—*Comparison of afternoon-night and forenoon trap catches in test and control mango trees without the presence of the repellent spray, expressed as log ratios*

Uniformity trial	Afternoon-night	Forenoon	Total
1	0.00	0.12	0.12
2	−.09	−.03	−.12
3	−.21	.16	−.05
4	−.03	−.06	−.09
Total	−.33	.19	−.14

ANALYSIS OF VARIANCE

Source of variation	Degrees of freedom	Sum of squares	Variance
Trial	3	0.0172	0.0057
Time of day	1	.0338	.0338
Error	3	.0441	.0147
Total	7	.0951	

The variation due to time of day is shown to be considerably less than significant.   The variation that does exist was acting to reduce the measurable effect of the repellent rather than to increase it, since the value of log R tended to be more positive in the forenoon than in the afternoon, whereas the application of the repellent produced a value that was negative in the forenoon and became more positive in the afternoon..   While more uniformity trials would have been desirable, there is no evidence that the observations attributed to the action of the repellent could have been due to natural variation alone.

PROTECTION OF FRUIT

After the first week in May some evidence developed that the repellent spray might be providing some protection against infestation. Therefore, the weekly spray program was continued to the end of June to compare the infestations of mangoes from the two trees. During June the fallen fruit was counted and examined.   On June 30 a large portion of the fruit was picked from the treated tree, and a sample from the control tree was held for comparison.   A summary of the fruit records is given in table 5.

TABLE 5.—*Comparison of infestation in fruits from sprayed and control mango trees, 1941*

FALLEN FRUITS

Date	From sprayed tree		Doubtful, between trees		From control tree	
	Total	Visibly infested	Total	Visibly infested	Total	Visibly infested
	Number	*Number*	*Number*	*Number*	*Number*	*Number*
May 30–June 3	8	5	8	3	19	14
June 9	10	7	9	7	30	22
June 17	39	30	22	15	65	37
June 24	37	29	12	12	45	39
June 30	55	46	21	18	120	78
Total	149	117	72	55	279	190
Percent visibly infested		78.5		76.4		68.1

PICKED FRUITS

Side of tree	Sprayed tree			Control tree		
	Total	Infested		Total	Infested	
	Number	*Number*	*Percent*	*Number*	*Number*	*Percent*
East:						
7–10 feet	176	41	23.3	110	60	54.5
10–14 feet	46	13	28.3			
South	99	57	57.6			

The experiment was not designed in such a way that a conclusive result could be expected.   The spray coverage was only fair.   The differences observed generally indicate less infestation in the sprayed tree.

The fallen fruit was examined every 3 or 4 days, but the data were combined in the table.   There is little difference in the percentage of visible infestation in fallen fruit between the sprayed tree and the control tree, because the fruit that falls in Cuernavaca is nearly all

green and damaged by *Anastrepha ludens*.   Since previous experience has shown that more than 90 percent of the fruit falling in June and July is infested, if the fallen fruit had been held for later observation many of the mangoes that appeared sound would have been found to be infested.   Nearly twice as many fruits fell from the control tree as from the sprayed tree, and the difference was nearly as great for visibly infested fruits.

The picked fruit was held for 8 days at 23° to 25° C. before it was examined, which was 14 days after the last spray had been applied. The east side of the tree appeared to be less heavily infested than the south side.   The appropriate comparison indicates that the infestation of the fruit on the control tree was about twice that of the sprayed tree.

Another indication that the repellent spray provided some protection was the fact that the top of the test tree, where coverage was poor, supplied a large part of the fallen fruit.   On June 30, when the mangoes were picked, most of the fruit in the top of the tree had fallen but most of the fruit on the branches 10 to 14 feet high remained.   The groups of treated fruit were evenly colored, whereas groups of untreated mangoes generally showed spots of premature color due to infestation.

In March the sprayed tree produced a small early crop of mangoes which was almost completely infested.

During the spray program an occasional ripe mango was available for tasting.   Mangoes that had received as many as 10 sprays showed no off flavor, but those tasted at the end of the spray program were of poor flavor.   No injury to foliage was observed, but oil spots developed on the fruit.

SUMMARY

In previous tests with repellent sprays against the Mexican fruitfly (*Anastrepha ludens* (Loew)) in Mexico, it was found that traps in sprayed trees sometimes yielded more flies than comparable traps in unsprayed trees, although the repellent seemed to provide protection to the fruit.   An experiment was therefore undertaken to test this apparent contradiction.   Traps containing a fruitfly lure were set out in two mango trees, and one tree was sprayed about once a week from March 27 to June 30, while the other tree was left unsprayed.   The flies caught in the traps were counted before each spraying and at various intervals (usually 3 to 5, 9 to 11, and 27 to 29 hours) after spraying, and the ratios of the counts in the two trees were analyzed statistically.

The repellent action was found to pass through two phases in its effect on the trap catch—a negative phase following the application of the repellent, during which the catch in the sprayed tree was lower than in the unsprayed tree, and a positive phase in which the catch in the sprayed tree was above normal.

Uniformity trials run when the catches had returned to equilibrium after an application indicated that the differences were due to the action of the repellent and not to natural variation.

A comparison of infestation in fruits from the sprayed and the unsprayed tree showed that the spray probably provided some protection to the fruit.

CYTOPLASMICALLY INHERITED MALE-STERILITY IN SUGAR BEETS [1]

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INTRODUCTION

Male-sterility, which appears to be relatively common in beets (*Beta vulgaris* L.), may have different modes of inheritance. The present paper deals chiefly with male-sterility as inherited in cross-pollinated varieties of sugar beets bred for resistance to curly top (*1*).[2]

To explain the transmission of the male-sterile tendency from parent to offspring in these beets it is necessary to consider a cytoplasmic basis of inheritance (*12*). Literature reviews by Correns (*3*) and Sirks (*15*) reveal the interesting fact that male-sterility in cross-pollinated plants appears to be more commonly explained by cytoplasmic inheritance than by genic, or Mendelian, inheritance. According to mathematical calculations by Lewis (*6*), there may be an opportunity for survival of male-sterile plants in cross-pollinated populations if the male-sterility is inherited cytoplasmically, but the opportunity for survival is very poor if the inheritance is through either dominant or recessive Mendelian factors. Mendelian factors responsible for male-sterility have been frequently encountered in artificially inbred populations of several species of plants (*6*), but such factors do not appear to operate to any marked extent in open-pollinated populations.

In connection with cytoplasmic inheritance, several instances of genic effects that modify the cytoplasmic effects have been reported (*6*, *7*, *15*). In flax (*2*, *4*), a single gene apparently determines male-sterility with one type of cytoplasm but not with another. The inheritance of male-sterility in beets appears to be similar to that described in flax, although the evidence indicates the operation of at least two and possibly more Mendelian factors in beets. The operation of the Mendelian factors in beets also gives rise to semi-male-sterile types.

MATERIAL AND METHODS

In the investigations reported here, cytoplasmically inherited male-sterility was found in varieties and strains derived directly or indirectly from the sugar-beet variety U. S. 1 (*1*), which was the first of the curly-top-resistant sugar-beet varieties released by the United States Department of Agriculture. About 2 percent of the plants in this variety were observed to be male-sterile but female-fertile. Because

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of self-sterility, cross-pollination prevailed as in most commercial varieties of sugar beets. Mass selection was the principal method of improvement, selections being based largely on disease resistance, size of beet, and sugar content. Some of the mass selections from U. S. 1 showed no male-sterility, whereas others showed an increased percentage. About 6 percent of the beets were observed to be male-sterile in the variety U. S. 33, derived from seed produced from 100 beets selected from U. S. 1. Some strains derived by mass selection from U. S. 33 showed a further increased percentage of male-sterile beets.

The Munerati annual beet [3] and the curly-top-resistant strain 12c (*11*), which was not derived from the U. S. 1 variety, were used in the investigation of male-sterility not cytoplasmically inherited.

Intensive studies of breeding behavior were made possible by maintaining some of the parental beets for a period of years by asexual propagation (*10*).

Appropriate crosses were made by exchanging paper bags over the inflorescences in the greenhouse. Experience showed that contamination from foreign pollen in the greenhouse was extremely rare. Except for limited use of self-fertile types (*11*), most material was so strongly self-sterile that emasculation was deemed unnecessary in connection with hybridization work, regardless of whether the beets were also male-sterile. The completeness of the self-sterility was checked by observation through transparent Pyralin windows in the paper bags. In several instances the transmission of the R (*13*) color factor furnished an additional check on the completeness of the self-sterility. Nonemasculated $r\,r$ beets fertilized with pollen from homozygous $R\,R$ beets produced offspring all of which carried the R factor, showing that no selfing had taken place.

A limited number of crosses were made by open pollination in the field in connection with breeding work.

Populations for classification were grown either in the greenhouse or in the field, according to convenience and amount of seed available. To prevent contamination from old ungerminated seed, the greenhouse soil was always steam-sterilized before seed was planted. In the field, an occasional error was possible because of contamination by ungerminated beet seed from a previous beet-seed crop, which had remained in the soil for 5 or 6 years. This source of contamination was too small to affect the general conclusions, but it may explain occasional instances of nonconformity.

Environmental variability made classification work difficult. The variable nature of the semi-male-sterile types caused the most difficulty. Usually it was necessary to pull the beets as the classifications were made, although more critical classifications were made in a few instances by periodic observations of beets held in greenhouse pots for a period of about 3 weeks during flowering. Conflicting classifications of data obtained from observations made on different dates, and a limited amount of variability among the different flowers of a plant on any given date, showed that classifications for degree of semi-male-sterility were subject to considerable error. Strong degrees of semi-male-sterility were sometimes confused with complete male-sterility, and beets with lesser degrees of semi-male-sterility were not infrequently classified as apparently normal.

[3] Obtained from Dr. O. Munerati, Director, R. Stazione Sperimentale di Bieticultura, Rovigo, Italy.

THEORETICAL ASSUMPTIONS

Although several Mendelian factors may influence the degree of male-sterility and there may be some instability or variation of substances carried by the cytoplasm, most of the data here recorded may be explained by two Mendelian factors carried by beets having two kinds of cytoplasm.

Assuming two types of cytoplasm, namely, that carried by normal hermaphrodites and that carried by male-sterile beets, they will be indicated by the symbols N and S, respectively. It will be assumed that semi-male-sterile types also carry S cytoplasm but that their appearance is modified by two Mendelian factors, X and Z. Completely male-sterile beets will be assumed to be of constitution S $x x z z$. The possible constitutions of heterozygous semi-male-sterile beets are S $X x z z$, S $x x Z z$, and S $X x Z z$. There may be a difference between effects produced by the factors X and Z, but since there is no evidence for such a difference, semi-male-sterile beets of constitution S $X x z z$ will be considered indistinguishable from beets of constitution S $x x Z z$. Discussion of semi-male-sterile types will be largely limited to a consideration of a heterozygous condition with regard to the factors X and Z. Semi-male-sterile beets derived from seed borne by male-sterile S $x x z z$ females could not be homozygous for X or Z.

DESCRIPTION

COMPLETELY MALE-STERILE BEETS

The female-fertile but completely male-ster'le beets (S $x x z z$) bear white, empty anthers (fig. 1) and may usually be identified before the flowers open by cutting into well-developed buds. Limited observations indicated that pollen mother cells and apparently normal microspores were formed, but the microspores failed to develop and by the time the flowers opened they had largely disintegrated. No detailed study was made of the disintegration of the microspore cells.

SEMI-MALE-STERILE BEETS OF TYPE 1

Beets of semi-male-sterile type 1 (S $X x z z$ or S $x x Z z$) produce yellow anthers, which usually do not dehisce; or, if they dehisce, most of the pollen grains adhere to the anther. In most instances the pollen grains produced are small (fig. 2, B) and nonviable, but small environmental variations cause relatively large differences. Under unfavorable environmental conditions these beets may bear pale-yellow and almost completely empty anthers. Under certain environmental conditions, not yet well defined, these semi-male-sterile beets may bear white, empty anthers and thus be indistinguishable from completely male-sterile beets. Under exceptionally favorable environmental conditions they may produce anthers fairly well filled with pollen grains, some of which may be viable.

SEMI-MALE-STERILE BEETS OF TYPE 2

Offspring from male-sterile (S $x x z z$) females that produced too much pollen to be classed as semi-male-sterile type 1 were arbitrarily designated semi-male-sterile type 2 and considered to be of constitution S $X x Z z$. In some instances it was suspected that more than two genetic factors might be involved, but proof for such an assumption must await additional results. The appearance of beets of semi-

male-sterile type 2 was markedly affected by small environmental variations, and sometimes there was much variation in the amount of pollen abortion on a single beet. When these beets, believed to be carrying S cytoplasm, could not be distinguished from normal hermaphrodites, a knowledge of their female parentage proved to be a safe criterion by which to determine their constitution and predict their breeding behavior.
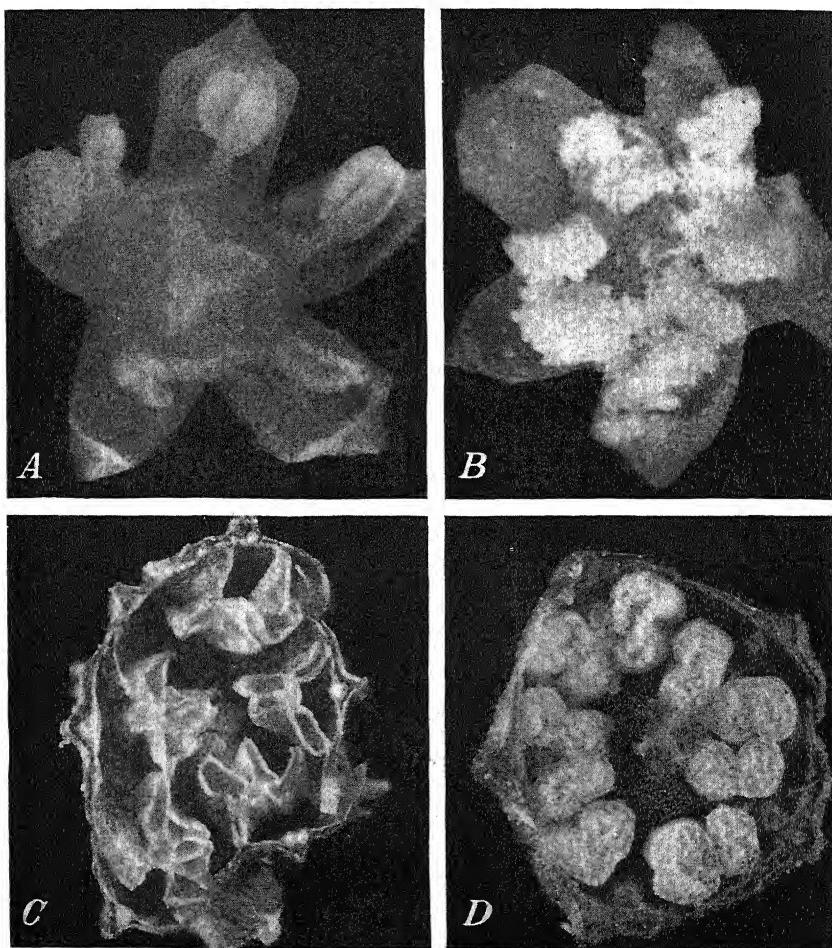
FIGURE 1.—Sugar-beet flowers. *A,* Male-sterile; × 12. *B,* Normal, immediately after dehiscing; × 12. *C,* Cross section of male-sterile flower in bud stage: × 21. *D,* Cross section of normal flower in bud stage; × 21.

EXPERIMENTAL RESULTS

RECIPROCAL CROSSES BETWEEN SEMI-MALE-STERILE AND SELECTED HERMAPHRODITE BEETS

If a character is to be explained by Mendelian, or genic, inheritance, the male and female parents may both influence the characteristics of the offspring, and populations obtained from reciprocal crosses should

be identical.   If a character is to be explained by cytoplasmic, or maternal, inheritance, the male parent may have no influence on the characteristics of the offspring, and populations obtained from reciprocal crosses should not be identical.   If cytoplasmic and genic
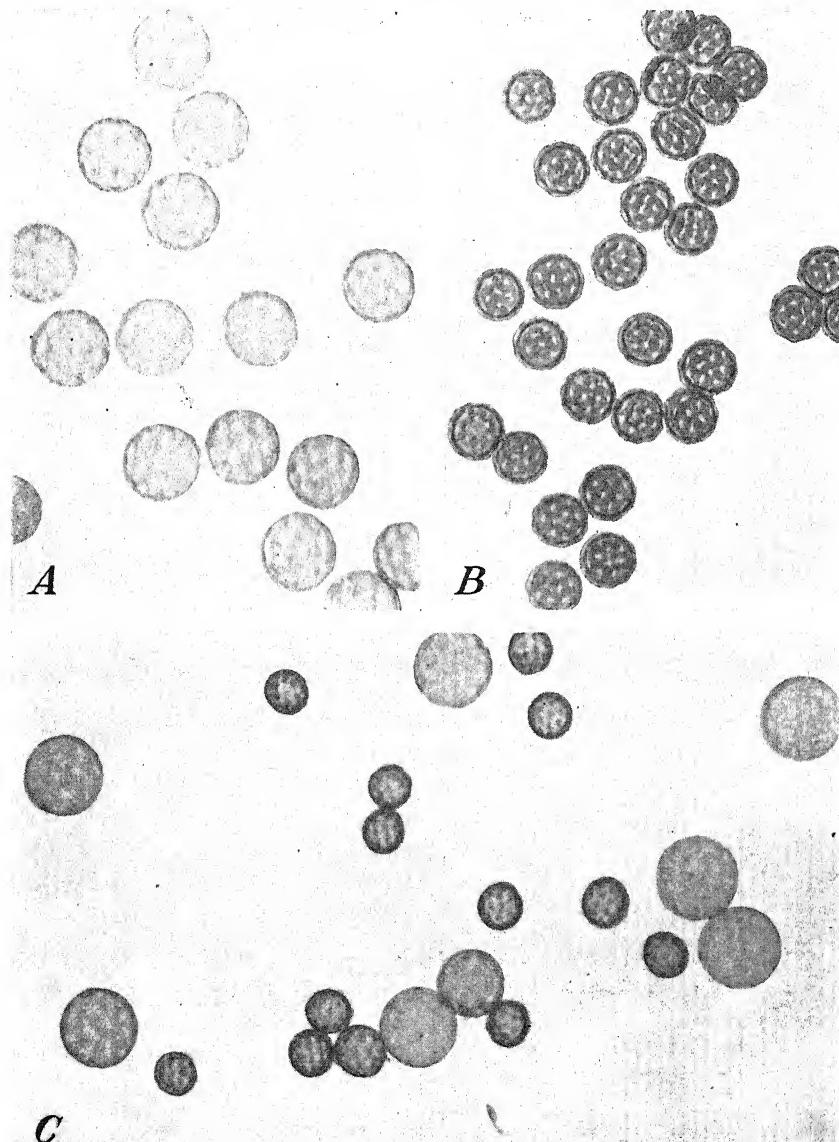
FIGURE 2.—Sugar-beet pollen: *A*, Normal; *B*, pollen from semi-male-sterile beet of type 1, with all pollen grains small and empty; *C*, pollen from semi-male-sterile beet of type 2, showing both normal and small, empty pollen grains. × 460.

inheritance are both involved in the expression of a character, the results are more complicated; but the female parent should exert more

influence than the male parent on the characteristics of the offspring, and populations obtained from reciprocal crosses should not be identical.

Reciprocal crosses could not be made with completely male-sterile beets, which produced no pollen; but many of the semi-male-sterile beets produced viable pollen, and eight reciprocal crosses were made with them. These crosses were made between six different semi-male-sterile beets and four different self-sterile hermaphrodites. The six semi-male-sterile beets differed in degree of semi-male-sterility, but a clearer discussion of these differences can be given when the effects of Mendelian factors are considered. The four hermaphrodites were selected clones with known breeding behavior. When used as pollen parents in crosses with completely male-sterile beets, these clones produced offspring nearly all of which were completely male-sterile.

The results obtained from the reciprocal crosses (table 1) were strikingly different. All the offspring grown from seed borne by the hermaphrodites, like their maternal parents, were apparently normal. The populations grown from seed borne by the semi-male-sterile beets segregated for complete male-sterility and semi-male-sterility, and in five of the populations a smaller percentage of the individuals were classified as apparently normal. This extreme difference between reciprocal crosses constitutes strong evidence for cytoplasmic inheritance. The complexity arising from the segregation in populations obtained from seed borne by the semi-male-sterile beets may be explained by assuming additional modifying effects produced by Mendelian factors.

TABLE 1.—*Reciprocal crosses between semi-male-sterile and selected hermaphrodite beets*

Parents [1]	Offspring			
	Male-sterile	Semi-male-sterile	Apparently normal	Total
	Percent	*Percent*	*Percent*	*Number*
A (semi-male-sterile) × clone 181	88	12	0	52
Reciprocal	0	0	100	111
B (semi-male-sterile) × clone 97	33	63	4	137
Reciprocal	0	0	100	43
C (semi-male-sterile) × clone 97	24	56	20	89
Reciprocal	0	0	100	15
C (semi-male-sterile) × clone 178	49	32	19	47
Reciprocal	0	0	100	22
C (semi-male-sterile) × clone 181	25	53	22	60
Reciprocal	0	0	100	84
D (semi-male-sterile) × clone 179	68	32	0	71
Reciprocal	0	0	100	74
E (semi-male-sterile) × clone 181	26	74	0	61
Reciprocal	0	0	100	119
F (semi-male-sterile) × clone 179	46	47	7	81
Reciprocal	0	0	100	41

[1] In presenting proof for cytoplasmic inheritance it is not necessary to consider the effects of two Mendelian factors assumed to be X and Z. However, the semi-male-sterile beet A was considered of constitution S $X x z z$ (or S $x x Z z$) while the semi-male-sterile beets B to F were considered of constitution S $X x Z z$. The four hermaphrodites (clones 97, 178, 179, and 181) carrying normal cytoplasm were considered of constitution N $x x z z$.

HYBRIDS BETWEEN MALE-STERILE AND HERMAPHRODITE BEETS

In preliminary tests, 91 different hermaphrodites selected at random were crossed to male-sterile (S $x x z z$) females. F_1 populations from 27 of the 91 hermaphrodites were completely male-sterile, indicating that

these 27 hermaphrodites might be of constitution N $x x z z$. Definite segregation was observed in populations derived from hybridization of the male-sterile females with the remaining 64 hermaphrodites. Semi-male-sterile offspring were conspicuous in all of these populations, indicating that the male parents carried one or more Mendelian factors that influenced the effect of the S cytoplasm. The results indicated that the constitution of 31 of the parental hermaphrodites may have been $S X x z z$ (or $S x x Z z$), that of 25 may have been $S X x Z z$, that of 4 may have been $S X X z z$ (or $S x x Z Z$), and that of 4 may have been $S X X Z z$ (or $S X x Z Z$).

Four beets, clones 97, 178, 179, and 181, which from their breeding behavior appeared to be of constitution N $x x z z$, were selected for intensive study and were maintained by vegetative propagation for a period of years. Eighteen crosses were made to male-sterile beets $(S x x z z)$ with clone 97; of 841 offspring observed, all were completely male-sterile (see table 3). Fourteen crosses were made to male-sterile beets with clone 178; of 525 offspring observed, all were completely male-sterile except 4 that were semi-male-sterile. Six crosses were made to male-sterile beets with clone 179; of 442 offspring observed, all were completely male-sterile except 3 that were semi-male-sterile. The exceptional semi-male-sterile beets observed among the offspring from clones 178 and 179 were so rare that they might be accounted for by contamination. Exceptional offspring from clone 181, however, appeared to be too numerous to be accounted for by contamination; so the complete results are presented (table 2).

Environmental conditions apparently had a marked influence on the expression of the semi-male-sterile condition, because in population 0503 (table 2) 18 percent were classified as semi-male-sterile in 1940, but under slightly different environmental conditions in 1941 all offspring appeared to be completely male-sterile. Some of the segregation for semi-male-sterility might be accounted for if some of the male-sterile females crossed with clone 181 were of constitution $S X x z z$ (or $S x x Z z$) instead of $S x x z z$ as expected. However, this assumption could not fully explain the occurrence of semi-male-sterile beets and apparently normal beets in populations 8543 and 8550 (table 2) because, in crosses with the same females, clone 97 produced offspring all of which were completely male-sterile. This evidence may indicate that clone 181 carried a Mendelian factor not carried by clone 97 and having a smaller effect than X or Z.

SUCCESSIVE BACKCROSSES TO SELECTED HERMAPHRODITE BEETS

Successive backcrosses made by applying pollen of clone 97 to male-sterile $(S x x z z)$ offspring continued to produce completely male-sterile populations (table 3). These decisive results were to be expected from the theoretical considerations, assuming clone 97 to be of constitution N $x x z z$.

It is of incidental interest that in the successive backcrosses the male-sterile offspring continued to be fully compatible with pollen from clone 97. This compatibility was also observed in 10 additional fourth-generation backcrosses made in 1943 and not shown in table 3. Insofar as they were tested, none of the male-sterile offspring showed any tendency to incompatibility with pollen from the parental clone 97. Clone 97, however, was strongly self-sterile. If a single series of allelomorphs determined self- and cross-sterility, some of the offspring

TABLE 2.—*Hybrids between male-sterile beets and clone 181*

Population No.	Offspring			
	Male-sterile	Semi-male-sterile	Apparent-ly normal	Total
	Percent	*Percent*	*Percent*	*Number*
8540	66	17	17	6
8543	76	14	10	21
8550	67	20	13	15
8572	100	0	0	20
8574	100	0	0	18
8622	100	0	0	15
9290	92	8	0	38
9471	98	2	0	52
0102	98	2	0	60
0103	94	6	0	54
0104	94	6	0	48
0105	98	2	0	64
0483	97	3	0	39
0485	100	0	0	52
0486	100	0	0	59
0503 [1]	82	18	0	34
0503 [2]	100	0	0	51
0511	100	0	0	35
0516	99	1	0	80
0519	100	0	0	34
0521	98	2	0	53
0533	95	3	2	57
0547A	100	0	0	45
0559	88	12	0	40
0574	92	8	0	62
0576	98	2	0	58
0579	91	9	0	54
0587	100	0	0	16
0589	97	3	0	60
0591	92	8	0	24
0593	87	13	0	54
0600	100	0	0	30
0603	100	0	0	33
0604	100	0	0	42
0607	98	2	0	42
1–559	100	0	0	105
Total observed	95.9	3.7	.4	1,570
Total expected	100	0	0	1,570

[1] Classified in 1940.
[2] Classified in 1941.

should have been incompatible with pollen from clone 97 in the second, third, and fourth backcross generations. That this incompatibility

TABLE 3.—*Hybrids between male-sterile beets and clone 97*

Generation	Popula-tions	Offspring		
		Male-sterile	Semi-male-sterile	Apparently normal
	Number	*Number*	*Number*	*Number*
F[1]	8	405	0	0
First backcross	3	158	0	0
Second backcross	4	177	0	0
Third backcross	3	101	0	0
Total	18	841	0	0

was not observed confirms previous evidence (*11*) that more than one allelomorphic series of factors is necessary to explain cross-sterility in beets.

HYBRIDS BETWEEN SEMI-MALE-STERILE AND SELECTED HERMAPHRODITE BEETS

From populations derived by crossing semi-male-sterile beets of type 1 (S X x z z or S x x Z z) with clone 97 (N x x z z), an excess of male-sterile offspring was obtained as compared with semi-male-sterile offspring when a 1:1 ratio was expected (table 4). Heritable factors may have caused the lack of agreement between expected and observed results, but environmental influences may be given first consideration. Under slightly different environmental conditions and with provision for closer examination, some of the apparently complete male-sterility may have been more accurately classified as semi-male-sterility. In 1 population of 71 beets (population 1–647, table 4), grown in the greenhouse to permit close periodical observations, a good agreement with the expected 1:1 ratio was observed, 49 percent being male-sterile and 51 percent semi-male-sterile.

Apparently normal offspring were observed in populations 2628 and 2631 (table 4), which would indicate female parentage of genic constitution X x Z z, rather than X x z z or x x Z z as expected from their appearance. The two semi-male-sterile female parents in question were from inbred lines with reduced vigor, which may account for their type 1 (S X x z z or S x x Z z) appearance but type 2 (S X x Z z) breeding behavior (see table 5).

TABLE 4.—*Hybrids between semi-male-sterile beets and clone 97*

Hybrid and population No.	Offspring			
	Male-sterile	Semi-male-sterile	Apparently normal	Total
S X x z z (or S x x Z z) × N x x z z:	*Percent*	*Percent*	*Percent*	*Number*
4325	92	8	0	75
5502	87	13	0	158
1–501	95	5	0	199
0497	85	15	0	47
1–629	76	24	0	68
1–631	90	10	0	68
1–647	49	51	0	71
1–668	68	32	0	76
1–669	70	30	0	77
1–682	59	41	0	164
1–696	86	14	0	70
2619	86	14	0	35
2628	53	33	14	36
2631	41	56	3	59
2635	94	6	0	33
Total	76.8	22.7	0.6	1,236
Expected 1:1 ratio	50	50	0	1,236
S X x Z z × N x x z z:				
1–648	60	38	2	55
1–649	33	63	4	137
1–685	43	41	16	63
1–686	24	56	20	89
1–693	58	37	5	79
2580	37	63	0	19
Total	40	60		442
Expected 1:3 ratio	25	75		442

From populations derived by crossing beets of semi-male-sterile type 2 (S X x Z z) with clone 97 (N x x z z), the observed results are in relatively close agreement with expected results (table 4), considering the nature of the environmental variability. The excessive number of individuals classified as completely male-sterile might be explained by environmental influence or by failure to make sufficiently

close examinations. It was sometimes difficult to distinguish semi-male-sterile beets from apparently normal hermaphrodites, so no close distinction should be made between these two classes. Both of these classes were expected, and furthermore the difficulty in distinguishing between them was expected.

Beets of semi-male-sterile types 1 and 2 were crossed with pollen from the additional hermaphrodite (N $x x z z$) clones 178, 179, and 181, and the results were similar to those obtained when clone 97 was used as the pollen parent. Observations of offspring from one of the hybrids with a semi-male-sterile (type 1) female and from five hybrids between semi-male-sterile (type 2) females and clones 178, 179, or 181 are shown in table 1 in connection with the presentation of results with reciprocal crosses.

HYBRIDS BETWEEN MALE-STERILE BEETS AND CLONE 90

The preliminary results indicated that the hermaphrodite designated clone 90 might be homozygous, with a constitution represented by S $X X z z$ or S $x x Z Z$. Observations from eight F_1 populations were eventually obtained after eight different crosses between male-sterile females and clone 90 had been made.

All the F_1 offspring were expected to be semi-male-sterile because they were assumed to be of constitution S $X x z z$ or S $x x Z z$. These were essentially the results obtained in seven of the eight populations (table 5). Under favorable environmental conditions the beets in these seven populations were very nearly uniform in degree of semi-male-sterility. The anthers were yellow, but very little pollen dehisced and none appeared to be viable. In population 2522 (table 5), 43 beets were classified and discarded by May 6, 1943. These were very nearly uniform in degree of semi-male-sterility. By May 24, 1943, during a period with higher temperatures, 15 more beets in this population came into flower. The degree of male-sterility of all these 15 beets was definitely more pronounced, 4 of them being completely male-sterile, with white anthers.

TABLE 5.—*Hybrids between male-sterile beets and clone 90*

Hybrid and population No.	Offspring			
	Male-sterile	Semi-male-sterile [1]	Bearing some pollen	Total
S $x x z z \times$ N $X X z z$ (or N $x x Z Z$):	*Percent*	*Percent*	*Percent*	*Number*
4344	11	89	0	9
4634	8	92	0	73
8568	7	93	0	15
1–566	24	68	8	74
1–573	8	92	0	63
2466	3	97	0	30
2522	7	93	0	58
2562	2	98	0	54

[1] Nearly all the semi-male-sterile offspring were very nearly uniform in appearance. The anthers were yellow and partially filled, but they seldom dehisced and apparently bore no viable pollen.

Results with population 1–566 (table 5) failed to confirm the results obtained with the other seven populations. Observations of 74 beets grown from this population showed that 24 percent were completely male-sterile, 68 percent were semi-male-sterile, and 8

percent produced more or less normal pollen. Environmental conditions were favorable for flowering and pollen development, so the cause of the relatively wide segregation in population 1–566 was believed to be heritable rather than environmental. To explain these exceptional results, it would be necessary to assume that the male-sterile (S x x z z) female parent carried two additional Mendelian factors, one increasing and the other decreasing the degree of male-sterility.

HYBRIDS BETWEEN MALE-STERILE AND SEMI-MALE-STERILE BEETS

It has been assumed that semi-male-sterile beets carry the same cytoplasm as completely male-sterile beets. The difference between male-sterile and semi-male-sterile beets is attributed to one or more Mendelian factors carried by the semi-male-sterile plants. If this assumption is correct, the Mendelian factors in question should be carried by any viable pollen produced by the semi-male-sterile plants. Consequently, a male-sterile (S x x z z) female crossed with any type of semi-male-sterile beet should produce some semi-male-sterile offspring. In no case should all the offspring be completely male-sterile, indicating male parentage of genic constitution x x z z.

Two crosses of the type S x x z z $\times$ S X x z z were made with the poor pollen from semi-male-sterile beets of type 1. Six crosses were made between male-sterile (S x x z z) beets and semi-male-sterile beets considered to be of type 2 and of constitution S X x Z z (table 6).

Since each of the eight populations (table 6) segregated for complete male-sterility and semi-male-sterility, the observed results are in essential agreement with expected results. The recovery of some apparently normal beets from the crosses with semi-male-sterile beets of type 2, used as pollen parents, was to be expected. If these apparently normal beets had been subjected to further study over a period of time, it is possible that they would have shown a tendency to semi-male-sterility.

TABLE 6.—*Hybrids between male-sterile and semi-male-sterile beets*

Hybrid and population No.	Offspring			
	Male-sterile	Semi-male-sterile	Apparently normal	Total
S x x z z $\times$ S X x z z (or S x x Z z):	*Percent*	*Percent*	*Percent*	*Number*
335	63	37	0	19
1–549	57	43	0	14
Total	61	39	0	33
Expected 1:1 ratio	50	50	0	33
S x x z z $\times$ S X x Z z:				
1–538	62	36	2	117
1–583	22	54	24	72
1–607	24	72	4	100
1–610	53	41	6	32
1–618	42	34	24	157
2470	58	38	4	26
Total	42	58		504
Expected 1:3 ratio	25	75		504

SEMI-MALE-STERILE BEETS SELFED

Male-sterile and semi-male-sterile beets apparently carrying S cytoplasm were crossed with hermaphrodites carrying a factor S^f for

self-fertility. From results of breeding behavior, it was eventually revealed that six of the self-fertile hermaphrodites used as pollen parents could be considered of constitution N $X x Z z$ with regard to inheritance of male-sterility. These six self-fertile (N $X x Z z$) hermaphrodites were used as pollen parents in crosses with both male-sterile and semi-male-sterile females. Wide segregations were observed in F_1 populations. F_2 populations were obtained by selfing selected self-fertile F_1 beets that produced sufficient viable pollen. The results are presented in table 7, the F_2 populations being grouped according to the classification of their respective female grandparents.

TABLE 7.—*Offspring of selfed beets carrying S cytoplasm, with pollen grandparents considered of constitution N X x Z z and female grandparents as indicated*

Grandparents		Self-fertilized population No.	Offspring			
Female	Male (N $X x Z z$)		Male-sterile	Semi-male-sterile [1]	Bearing some pollen [2]	Total
Male-sterile (S $x x z z$):			*Percent*	*Percent*	*Percent*	*Number*
Group	9508–1	1–752	44	26	30	27
	9508–1	1–753	42	42	15	33
	9508–1	1–754	51	31	18	93
	9508–1	1–755	39	50	11	38
	0448–48	2267	39	49	12	43
	0448–48	2268	20	70	10	110
9148–4	0448–48	2269	27	29	44	52
	0448–48	2270	20	36	44	50
	0448–48	2271	40	47	13	15
	0448–48	2272	14	48	38	21
0579–151	0448–48	2273	26	31	43	35
Semi-male-sterile (S $X x z z$ or S $x x Z z$):						
8114–105	0475–13	2263	7	93	0	28
	0475–15	2264	12	88	0	83
	0475–20	2265	0	90	10	99
	0475–20	2266	20	80	0	41
046–1606	0475–20	2274	15	65	20	106
	0475–20	2275	2	60	38	105
Semi-male-sterile (S $X x Z z$):						
046–1121	0475–20	2276	0	38	62	69
	0475–20	2277	7	49	44	105
	0475–20	2278	0	79	21	123
	0475–20	2279	1	70	28	67
0107–4	9254–1	2280	0	24	76	25
0493–1	0475–13	2281	0	0	100	58

[1] Offspring classified as semi-male-sterile bore yellow anthers but apparently no viable pollen.
[2] Some offspring classified as bearing pollen showed evidence of being semi-male-sterile, but no clear distinction could be made between this condition and the normal hermaphrodite condition.

In the first group (table 7), where the grandparents were considered to be S $x x z z$ and N $X x Z z$, all 11 of the observed self-fertilized populations showed wide segregation, some beets being completely male-sterile, some semi-male-sterile, and others relatively normal. In the second group, where the grandparents were considered to be S $X x z z$ (or S $x x Z z$) and N $X x Z z$, all 6 of the observed self-fertilized populations showed segregation, but the average percentage of male-sterile beets was less than in the first group. In the third group, where the grandparents were considered to be S $X x Z z$ and N $X x Z z$, the 6 observed self-fertilized populations showed definitely less segregation, population 2281 showing no segregation. The beets in population 2281 were uniform with regard to pollen production. The amount of pollen was perhaps less than normal, but microscopic examinations failed to show any consistent difference between the pollen from these beets and that pollen produced by normal hermaphrodite beets.

Crosses have been made between male-sterile (S $x x z z$) females and self-fertile beets considered to be of possible constitution S $X X Z Z$, like the self-fertile beets in population 2281. From these crosses it is expected that more information may be obtained regarding the Mendelian factors that influence the degree of male-sterility in the presence of S cytoplasm.

<div style="text-align:center">OPEN POLLINATIONS</div>

As a rule the most critical genetic information is obtained from controlled crosses where the male parent, as well as the female parent, is known. With cytoplasmic inheritance, however, it is possible to obtain critical information regarding type of cytoplasm by open pollination to a heterogeneous source of pollen.

Seven apparently normal beets grown from seed borne by male-sterile (S $x x z z$) females were investigated to determine whether they carried S cytoplasm as expected. These seven beets were grown in three different beet-seed isolations. They showed no sign of semi-male-sterility during repeated examinations. Each beet was tagged for identification during flowering and was allowed to open-pollinate with the mass of unknown pollen to which it was exposed. The results from the populations grown from the open-pollinated seed borne by the seven apparently normal beets are shown in table 8. All seven of the populations produced male-sterile and semi-male-sterile offspring as well as variable percentages of apparently normal offspring. This is ample proof that the seven apparently normal female beets carried S cytoplasm. If they had carried N cytoplasm, all the offspring should have been normal.

TABLE 8.—*Offspring derived by open pollination from apparently normal beets carrying S cytoplasm*

Population No.	Offspring			
	Male-sterile	Semi-male-sterile	Apparently normal	Total
	Percent	*Percent*	*Percent*	*Number*
0111	39	37	24	38
1-117	27	24	49	66
1-118	37	53	10	90
1-119	18	58	24	91
1-120	6	60	34	67
1-135	14	64	22	92
1-136	54	39	7	56
Total	26	50	24	500

<div style="text-align:center">CYTOPLASMIC INSTABILITY</div>

Substances carried by the cytoplasm and responsible for cytoplasmic inheritance appear to be relatively stable in some instances and relatively unstable in others. When a chlorophyll deficiency is brought about by cytoplasmic inheritance it is not uncommon in several species of plants (*15*) to see sectorial arrangements, some branches being green, others white, and others partly green and partly white. An unstable chlorophyll condition of this type, believed due to cytoplasmic inheritance, has been described in beets (*8*, *13*). After extensive investigations with cytoplasmic inheritance

in *Epilobium*, Michaelis (*7*) stated that the substances carried by the cytoplasm that are responsible for cytoplasmic inheritance are much less stable than the genes carried by the chromosomes. A somewhat different opinion is expressed by Lewis (*6, p. 61*), as follows:

> Cytoplasmic mutations appear to occur more rarely than gene mutations; however, it is probable that changes in the cytoplasm arise more frequently than the data reveal, but are not observed because the effects are so slight.

The writer's observations would indicate that the cytoplasmically inherited male-sterility in beets is a relatively stable character. However, some unexplained results might be accounted for by cytoplasmic instability, and in 2 instances the instability was definite and of striking degree. In the course of routine classification work, 2 beets from rather widely separated parentage were observed to be highly variable. Most of the inflorescence of each of these beets showed complete male-sterility, but some sectors showed semi-male-sterility, occasional anthers producing viable pollen. One of these variable beets was self-fertile, and observations were continued by growing its offspring with population 2612, obtained from self-pollinated seed, and with population 2613, obtained from open-pollinated seed produced by the same plant. Twenty-six offspring were grown from population 2612 and 38 from population 2613. All these offspring were observed to be completely male-sterile, except 9 individuals (2 from population 2612 and 7 from population 2613) which exhibited an unstable condition similar to that of the maternal parent. On these 9 offspring, viable pollen was produced by some branches of inflorescences, although most of the branches bore anthers that were white and completely male-sterile. Variability upon single branches was also observed, and in a few instances empty, white anthers and yellow anthers filled with pollen were borne within the same flower.

The maternal beet of populations 2612 and 2613 might be considered of genic constitution $x\,x\,z\,z$ because of the high proportion of complete male-sterility observed in the offspring. This parental beet and those of the offspring also showing the unstable condition evidently carried S cytoplasm, but the evidence indicates that it was not the usual kind of S cytoplasm. Apparently some change in its stability had taken place. The sectors bearing viable pollen might be considered of constitution N $x\,x\,z\,z$, whereas most of the male-sterile portion of the beet might be considered of constitution S $x\,x\,z\,z$.

MALE-STERILITY NOT CYTOPLASMICALLY INHERITED

Male-sterility apparently similar to that already described but with a different mode of inheritance was encountered in two different varieties of beets. One variety was the 12c strain (*11*), a noncommercial curly-top-resistant variety, and the other was the Munerati annual variety (*9*). Both of these varieties had apparently been derived by relatively close breeding, and occasional male-sterile beets were observed in both.

Two male-sterile beets, 841–9 and 841–10, from the Munerati annual variety were crossed with the well-proved N $x\,x\,z\,z$ hermaphrodite clones 97 and 179. A male-sterile beet designated 3252–276, from the 12c strain, was crossed with pollen from clone 97. All the offspring (table 9) appeared to be normal hermaphrodites with a heavy production of pollen and without any indication of male-

sterility or semi-male-sterility. If the male-sterile female parents had carried S cytoplasm, all the offspring should have been male-sterile. The evidence, therefore, indicates that the male-sterility in the 12c strain and in the Munerati annual variety must have been produced by a different mode of inheritance. This inheritance may have been entirely genic, without cytoplasmic influence.

TABLE 9.—*Hybrids between exceptional male-sterile and selected hermaphrodite beets*

Population No.	Male-sterile female	N x x z z normal male	Offspring		
			Male-sterile	Semi-male-sterile	Apparently normal
			Number	*Number*	*Number*
2855	841-9	Clone 97	0	0	21
2856	841-9	Clone 179	0	0	19
2859	841-10	do	0	0	21
4789	3252-276	Clone 97	0	0	9

In inbred lines of beets, the writer has observed additional instances of male-sterility where the character is probably independent of cytoplasmic inheritance. Male-sterility has been common in inbred lines, even where there was good evidence that the lines carried N rather than S cytoplasm.

UTILIZATION OF MALE-STERILITY IN HYBRIDIZATION WORK

The cytoplasmically inherited male-sterility in beets offers unique possibilities for obtaining a wholesale emasculation of flowers to facilitate hybridization work. Controlled crosses make it possible to produce male-sterile offspring in whatever quantity is desired, either for small-scale genetic studies or for field crosses for the production of hybrid seed. It has been shown that, with few exceptions, crosses of the type S x x z z × N x x z z produce offspring all of which are completely male-sterile. Therefore, obtaining a quantity of seed that can be relied upon to produce completely male-sterile offspring is dependent upon selecting a sufficient number of hermaphrodites that may be considered of constitution N x x z z.

Hermaphrodites that may be considered of constitution N x x z z appear to be fairly common in curly-top-resistant varieties of sugar beets, but they can be identified only by breeding behavior. The N x x z z hermaphrodites were propagated in three ways: (1) By clonal or asexual propagation (*10*), (2) by introducing a factor for self-fertility (*11*) and inbreeding, and (3) by reproducing offspring obtained by intercrossing self-sterile N x x z z clones with proved breeding behavior. Vegetative propagation alone has only limited applications, although such propagation helps materially in holding clones until their breeding behavior is known. The inbreeding method has great potentialities, but inbred lines of sugar beets with satisfactory resistance to curly top are only now beginning to be made available. Therefore, the third method, namely, working with intercrosses between self-sterile beets that may be considered of constitution N x x z z has been the principal method of propagation used by the writer.

The self-sterile clone 97, whose breeding behavior indicated the N x x z z constitution, was hybridized with other self-sterile (N x x z z) clones with similar breeding behavior. The self-sterile F₁ populations

were reproduced by maturing seed by open pollination in isolated field plantings. Hybrids between selected male-sterile (S x x z z) beets and the N x x z z populations made it possible to obtain an abundance of seed that could be depended upon to produce completely male-sterile offspring with but very few exceptions. The exceptional offspring, although not completely male-sterile, generally produced no viable pollen. Finally a field planting was made in which 16-row strips of male-sterile beets were alternated with 4-row strips of a normal hermaphrodite variety (fig. 3). With this planting arrangement, pollination by insects or by wind appeared to be adequate because the acre yield of seed from the male-sterile strips was about 3,000 pounds, approximating or slightly exceeding the yield from the strips of the normal variety.


FIGURE 3.—Sugar-beet seed field in which 16-row strips of male-sterile sugar beets were alternated with 4-row strips of a normal variety. The color contrast was due to the male-sterile beets being a darker green than the normal pollen-bearing beets.

Excellent male-sterile breeding stock was produced in this manner, but no attempt is being made to hasten commercial applications for the production of hybrid seed until the underlying research work, including work with inbred lines, has made further progress.

DISCUSSION

The findings with respect to combined effects of cytoplasmic and genic inheritance make it apparent that caution should be used in attributing all the influence to either of these two modes of inheritance. This need for caution may be illustrated by the male-sterility in the curly-top-resistant sugar beets where male-sterile beets may be considered of constitution S x x z z, S representing a particular type of cytoplasm and x x z z representing the complementary genic constitution. Observations restricted to offspring from hybrids between male-sterile S x x z z females and N x x z z hermaphrodites would lead to the assumption of cytoplasmic inheritance without genic influence. Observations restricted to offspring derived by selfing beets of constitution S X x Z z, with the degree of male-sterility determined by the recombination of the Mendelian factors X and Z, would lead to the assumption of genic inheritance without cytoplasmic influence. A comprehensive understanding of the combined effect

of the two modes of inheritance can be gained only by devising tests for both the cytoplasmic inheritance and the genic inheritance.

It is of interest that in corn (*Zea mays*), although Mendelian factors for male-sterility have been frequently reported (*5*), cytoplasmically inherited male-sterility has not been reported except from limited sources in South America. Gini (*5*) investigated different sources and modes of inheritance of male-sterility in *Z. mays* found in Argentina and traced all the cytoplasmically inherited male-sterility to a single region, the Province of Entre Ríos. Rhoades (*14*) reported another case of cytoplasmically inherited male-sterility in the offspring of *Z. mays* collected at Arequipa, Peru.

SUMMARY AND CONCLUSIONS

Male-sterility produced by combined cytoplasmic and genic inheritance was found in cross-pollinated varieties of sugar beets bred for resistance to curly top. Complete male-sterility was characterized by white, empty anthers. The inheritance of semi-male-sterility was found to be related to that of complete male-sterility. Assuming two types of cytoplasm, S for male-sterility and N for normal, and two Mendelian factors X and Z, the majority of the evidence, including striking differences from reciprocal crosses, indicates the following constitution of male-sterile and heterozygous semi-male-sterile beets:

S x x z z = completely male-sterile.
S X x z z (or S x x Z z) = semi-male-sterile, usually without viable pollen.
S X x Z z = semi-male-sterile, usually with some viable pollen and sometimes indistinguishable from normal hermaphrodite.

Occasional results were not fully explained by the two-factor hypothesis, indicating that more than two Mendelian factors may influence the degree of semi-male-sterility. Instances of homozygosity for the Mendelian factors were demonstrated, but offspring from male-sterile (S x x z z) females were never found to be homozygous for X or Z.

The male-sterile character in the curly-top-resistant varieties of beets appeared to be relatively stable, but in two instances cytoplasmic instability was encountered, single male-sterile beets producing sectors of inflorescence bearing more or less normal anthers with viable pollen. Seed from beets in this unstable condition produced offspring some of which continued to show striking degrees of instability.

The male-sterile sugar beets produced by combined cytoplasmic and genic inheritance were found to be especially convenient for use as female parents in hybridization work, because the nature of the inheritance makes possible the production of completely male-sterile populations. With but very few exceptions, male-sterile (S x x z z) females crossed with selected N x x z z hermaphrodites produced completely male-sterile offspring.

Evidence indicates that some types of male-sterility in beets are produced by genic rather than cytoplasmic inheritance. Male-sterility found in the Munerati annual beet and in the curly-top-resistant strain 12c did not appear to be inherited cytoplasmically.

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RESISTANCE IN POTATO VARIETIES TO YELLOW DWARF [1]

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INTRODUCTION

The yellow dwarf disease of potato (*Solanum tuberosum* L.)was of little concern in Wisconsin before 1930, but during the years 1930, 1931, and 1932 it increased to such an extent in the central part of the State, especially in the counties of Portage, Waupaca, and Waushara, that it became the most important virus disease and in many cases the limiting factor in potato production. The disease was also very severe in Washington County in southeastern Wisconsin in 1935. In 1937 it was again of major importance in the State, not only in the central part but throughout Fond du Lac, Outagamie, Racine, and Washington Counties. Yellow dwarf is also present, although to a lesser extent, in the northwest section in Barron, Polk, and Washburn Counties and in the northeastern section in Door, Marinette, and Oconto Counties. The endemic occurrence and sporadic appearance of the disease renders it a constant hazard to the late potato crop. The loss sustained by the growers consists not only in decreased yields of crops grown for table stock but also in the added cost of replacing the virus-infected seed stock.

When infected seed stocks were recognized as the most important source of virus infection, the yearly planting of seed from yellow-dwarf-free areas was recommended as a control measure for central Wisconsin. The results were very encouraging when this practice was followed. However, the cost of seed is an important factor with the majority of small growers and the replanting of locally grown seed stock continued to be a common practice. This replanting of infected seed furnished a reservoir for yellow dwarf infection of other introduced virus-free potato stocks. Moreover, in many instances during a season of extensive spread of the virus, plants from disease-free seed accumulated enough of the virus to make the crop unprofitable.

Since the heavy losses in the zones where yellow dwarf commonly builds up rapidly could be materially reduced by the increased use of a late potato variety tolerant to the virus and adapted to the area, the existence of resistance in standard potato varieties has been studied since 1937. The first reference to the susceptibility of potato varieties to the yellow dwarf virus was made by Barrus and Chupp (*2*) [2] in New York in 1922. They observed the disease in 18 varieties of potatoes and reported that none of them showed any degree of resistance. Fernow and Black (*4*) in 1932 reported yellow dwarf more abundant in the Green Mountain than in the Rural variety. Muncie (*8*) in 1935 asserted that all commercial varieties of potatoes grown in

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Michigan were susceptible but made no mention of differences in disease incidence. A year later, Wheeler (*12*) reported that all the commercial potato varieties grown in Michigan showed a high percentage of the disease but that several potato-seedling varieties under test showed resistance to infection under natural field conditions and under artificial conditions by plug-graft inoculations. Black (*3*) in 1937 reported from New York that no immune potato plant had yet been discovered. In 1938, Taylor (*10*) found in a variety test planting in New York that the Columbia Russet variety was the most severely infected and Russet Burbank the least infected of 7 varieties when exposed to natural field infection. In a preliminary report on field resistance Walker and Larson (*11*) reported in 1939 that of 19 varieties or strains of potato that had been exposed to natural field infection in central Wisconsin, the Russet Burbank showed the lowest incidence of the disease. Hansing (*5, 6*), reporting in 1942 and 1943, stated that in several replicated field experiments conducted in New York the varieties Arran Banner, Chippewa, Golden, Houma, Jubel, Katahdin, Sebago, and Warba had less than 2 percent of yellow-dwarf-infected plants while Russet Burbank had 2 to 11, Rural 2.9 to 21.1, Russet Rural 4.2 to 29.1, Green Mountain 6 to 53, Early Bovee 18 to 28, and Red McClure 51.6 percent, respectively. He also stated that in 20 United States Department of Agriculture potato seedling varieties under test, the percentage of infected plants ranged from 0 to 72. Recently, Rieman and McFarlane (*9*) reported that yellow dwarf was more severe in Russet Rural than in Sebago during 1941.

EXPERIMENTAL RESULTS

FIELD EXPERIMENTS

1937–38 TRIALS

In 1937, 19 certified varieties or strains of potatoes were grown as an adaptation planting in a plot of 4 randomized replicated blocks situated 2 miles north of Almond, Portage County, Wis. This planting was one of the 7 potato variety adaptation plantings set up in the State that year by J. W. Brann, of the Departments of Horticulture and Plant Pathology of the University of Wisconsin. Pronounced primary yellow dwarf symptoms were observed about the middle of August on a large number of plants in this planting. The current-season symptoms were not confined to any one variety or strain but were scattered throughout the planting. A small percentage of secondarily infected plants were found in one of the varieties early in the season. This small amount of virus, however, was not entirely responsible for the pronounced spread of disease since the adaptation planting was surrounded on 3 sides by an 8-acre commercial field of Russet Rural potatoes in which 3 percent of the plants showed secondary yellow dwarf symptoms early in the season (fig. 1). It was observed during early September that the varieties in the adaptation planting exhibited marked variation in the incidence of primary yellow dwarf symptoms, although such differences could not be accurately recorded.

The spread of the yellow dwarf virus in the Almond area and in most of the central Wisconsin commercial potato section generally during the growing season of 1937 was very pronounced. A count of

yellow dwarf in the Russet Rural planting surrounding the adaptation planting in the latter part of the growing season showed primary symptoms on about 65 percent of the plants, and sweepings revealed
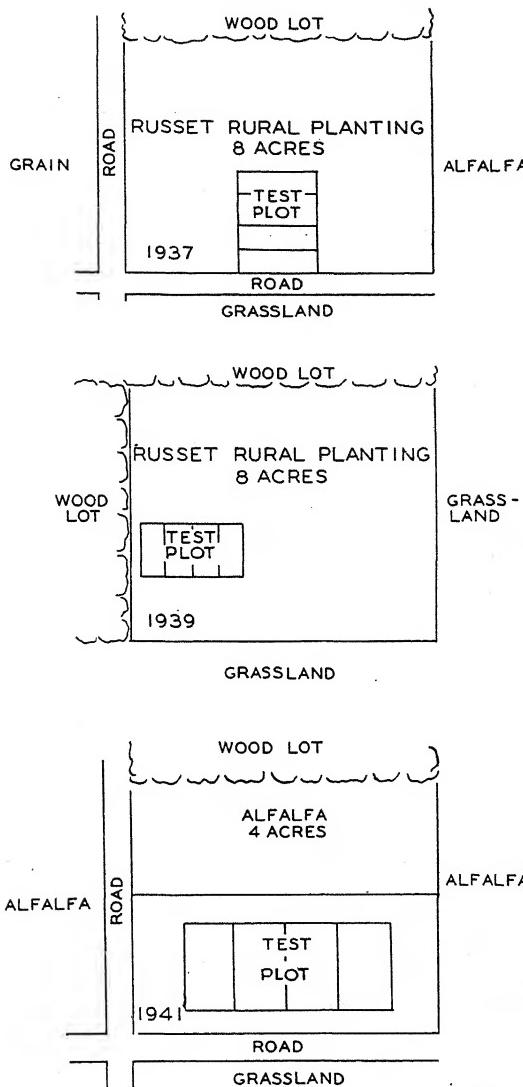
FIGURE 1.—Crop plantings of fields contiguous to replicated potato variety test blocks in a plot in central Wisconsin, near Almond, Portage County, 1937, 1939 and 1941.

a very large population of the clover leafhopper (*Aceratagallia sanguinolenta* (Prov.).

After harvest of the potatoes in the adaptation planting a random sample of U. S. No. 1 size tubers from a composite of the 4 replicates of each of the 19 varieties was placed in a common storage. A 40-tuber sample from each composite was obtained and planted as

wholetuber seed pieces at Hancock, Wis., in early May 1938. The detectable secondary yellow dwarf symptoms and nonemerging hills resulting from tuber infection were recorded and the tabulated results are presented in table 1.

It will be noted that the late-maturing Russet Burbank and the early-maturing Warba (pink-eyed) showed the lowest percentage when both nonemerging hills and secondary yellow dwarf symptoms are considered. Russet Rural and Triumph were the most severely infected of the late- and early-maturing varieties, respectively.

TABLE 1.—*A comparison of the incidence of the yellow dwarf virus as shown by secondary field symptoms during each succeeding season in certain varieties and strains of potato grown under natural field conditions in randomized replicate blocks in the high-disease zone near Almond, Wis., in 1937, 1939, and 1941*

Varieties under test	1937–38			1939–40			1941–42			1937–38, 1939–40, 1941–42		
	Nonemerging hills	Secondary yellow dwarf hills	Total nonemerging and secondary yellow dwarf hills	Nonemerging hills	Secondary yellow dwarf hills	Total nonemerging and secondary yellow dwarf hills	Nonemerging hills	Secondary yellow dwarf hills	Total nonemerging and secondary yellow dwarf hills	Average nonemerging hills	Average secondary yellow dwarf hills	Average total nonemerging and secondary yellow dwarf hills
	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.
Russet Rural	43	33	76	38	60	98	6	45	51	29	46	175xxx
White Blossom Cobbler [2]	68	5	73	----	----	----	----	----	----	68	5	73x
Triumph	53	28	81	20	76	96	4	32	36	26	45	71xxx
Toanco [3]	48	23	71	----	----	----	----	----	----	48	23	71x
Mesaba	30	40	70	----	----	----	----	----	----	30	40	70x
Green Mountain	23	47	70	4	66	70	8	47	55	12	53	65xxx
Earlaine	38	23	61	----	----	----	----	----	----	38	23	61x
Rural New Yorker	38	13	51	28	56	84	3	29	32	23	33	56xxx
Columbia Russet	13	38	51	----	----	----	----	----	----	13	38	51x
Pioneer Rural [4]	20	30	50	----	----	----	----	----	----	20	30	50x
Pontiac	----	----	----	----	----	----	0	44	44	0	44	44x
Katahdin	13	40	53	8	42	50	4	23	27	8	35	43xxx
Houma	15	38	53	0	32	32	----	----	----	8	35	43xxx
Chippewa	8	40	48	6	38	44	1	17	18	5	32	37xxx
Irish Cobbler	18	28	46	0	38	38	3	15	18	7	27	34xxx
One Hundred Day Cobbler	15	18	33	----	----	----	----	----	----	15	18	33x
Sequoia	----	----	----	----	----	----	5	19	24	5	19	24x
Russet Burbank	5	13	18	----	----	----	2	12	14	6	13	19x
Warba [5]	5	23	28	0	10	10	3	5	8	3	13	16xxx
Sebago	----	----	----	2	4	6	2	4	6	2	4	6xx

[1] x=1-year test; xx=2-year test; xxx=3-year test.
[2] Natural seedling of Cobbler.
[3] Strain of Rural New Yorker.
[4] Smooth mutant of Russet Rural.
[5] 1937–38, Pink-eyed Warba; 1939–40 and 1941–42, Red Warba.

1939–40 TRIALS

The opportunity for further studies of varietal resistance in the potato to the yellow dwarf virus under natural field conditions was again afforded during the season of 1939. An early-season examination of the large commercial planting (fig. 1) of the Russet Rural variety 1 mile south of Almond, surrounding the central Wisconsin varietal adaptation planting, was found to contain about 6 percent of secondary yellow dwarf plants, and the insect vector (*A. cerata-gallia sanguinolenta*) was present in considerable numbers. This potato variety adaptation planting was one of 9 field plantings set up

in 1939 at widely distributed points in the State by G. H. Rieman, of the Departments of Genetics, Horticulture and Plant Pathology, University of Wisconsin. The 10 varieties in the adaptation planting were either of certified seed or of foundation seed stock free of the yellow dwarf virus and were planted in 4 blocks in a plot in which the arrangement of varieties was randomized. Red Warba (7) was substituted in this trial for the pink-eyed Warba. A late-season count in the commercial Russet Rural planting surrounding the adaptation planting showed primary yellow dwarf symptoms on about 72 percent of the plants, indicating a very general spread of the virus in this planting. In the adaptation planting, primary yellow dwarf symptoms were observed throughout the replicated blocks.

A 50-tuber sample of U. S. No. 1 size from the composites of each variety in the adaptation planting were obtained and planted in 100-hill, single-row, 2-hill-per-tuber units at Almond in late May 1940. The occurrence of secondary yellow dwarf and of nonemerging hills were recorded in detail during the season. The data are presented in table 1.

When percentages of nonemerging hills and of secondary yellow dwarf were considered, Russet Rural and Triumph again showed the highest incidence of disease. Red Warba and Sebago were least affected. It is significant to note that there was again no correlation between time of maturity of variety and resistance, since the two most resistant varieties were in one case a very early maturing one (Warba) and in the other a very late maturing one (Sebago).

1941–42 Trials

Because of the differences in disease incidence exhibited by certain potato varieties in the two earlier field plantings, it was important to know whether varieties that showed low incidence of secondary yellow dwarf after being subjected to severe infection under natural conditions, would continue to show resistance when disease-free seed of these varieties was again exposed to severe natural virus infection.

In the spring of 1941 a test planting was made in the field previously used for the 1937 adaptation planting (fig. 1). However, 4 acres of alfalfa had replaced half of the Russet Rural planting area, and across the road alfalfa had replaced the grain. Twelve yellow-dwarf-free varieties (certified or foundation stock) were planted in 4 randomized blocks of 25 hills each. To insure as uniform infection as is possible under field conditions, 4 rows of yellow-dwarf-infected Russet Rural seed tubers (rows 2 to 5, 7 to 10, 12 to 15, etc.) were planted between each variety row; thus all test varieties were immediately adjacent to plants growing from virus-infected seed, as shown in figure 2. In addition, the entire test plot was surrounded by 30 rows of infected seed tubers. The yellow-dwarf-infected seed tubers planted as inoculum were selected in the spring of 1941 from a Russet Rural seed stock grown for two successive seasons in an area of central Wisconsin where yellow dwarf was prevalent. This seed stock was found to contain about 27 percent of secondary yellow-dwarf-infected plants early in 1940 and when examined later that season showed about 60 percent primary infection.

In the Almond area during the 1941 season insect sweepings in commercial potato fields, along roadsides, and fence rows, in grass-

lands and in waste areas showed a large population of the yellow-dwarf vector. A field count of primary disease symptoms in commercial potato plantings in this area and in the Russet Rural planting surrounding the test varieties indicated a pronounced current season spread of the virus. A late-season survey of the varietal test planting showed widespread current-season infection. At harvest a 300-pound random sample of U. S. No. 1 size tubers was collected from each composite of the four replicates of each variety grown in the test planting and placed in a common storage at Almond for the 1942 field planting.

FIGURE 2.—Planting arrangement of test varieties and yellow-dwarf-infected Russet Rural potatoes at Almond, Portage County, Wisc., 1941.

A random 200-tuber sample of each composite of the 12 varieties was selected in May 1942, and planted at Almond as whole-tuber seed pieces in 100-hill, 2-row plots, in early June. The nonemerging hills and detectable secondary yellow dwarf symptoms were recorded during the season and the data as to the rate of degeneration after infection in the 12 standard varieties under test are recorded in table 1.

The marked variation in incidence of disease between the varieties in the 1941–42 test is regarded as quite significant in view of the earlier field tests and the equal, uniform exposure of all varieties to natural infection by the yellow dwarf virus in the randomized arrangement in which the varieties were replicated in this test. Sebago, Red Warba, and Russet Burbank again showed a definite, high degree of field resistance to the yellow dwarf virus.

The season averages of secondary disease and nonemergence are presented in table 1. Measurable differences in varietal reaction to

the yellow dwarf virus are definitely apparent. Russet Rural, Green Mountain, and Triumph are distinctly the most susceptible. Sebago, Warba, and Russet Burbank show a definite field resistance; and Rural New Yorker, Katahdin, Chippewa, and Irish Cobbler are intermediate in their resistance. In less exhaustive tests White Blossom Cobbler, Toanco 4, Mesaba, and Earlaine show field susceptibility, and Columbia Russet, Pioneer Rural, Pontiac, Houma, One Hundred-Day Cobbler, and Sequoia are additional intermediate resistant varieties.

In addition to the tests in 1940 and 1942, a commercial field planting conducted in the Almond area with the extra tubers of the Sebago seed stock that had been exposed to yellow dwarf infection in the 1939 adaptation planting showed little or no increase of the virus over a period of 3 years. When the Sebago tubers not used in the test planting in 1940 were increased, the percentage of disease was the same (6 percent) as in the 1940 test plot. In a 1941 planting of this same Sebago seed stock, only 8.5 percent of the plants showed secondary yellow dwarf late in the season. In a planting of the same Sebago seed stock again in 1942, only 5.5 percent of secondary yellow dwarf was observed, showing no increase over the 1940 and 1941 plantings. The high degree of resistance of the late-maturing Sebago variety under field conditions in the portion of central Wisconsin where yellow dwarf is very destructive is clearly evident.

CLOVER LEAFHOPPER POPULATION

No insect vector counts were made during the seasons of 1937 and 1939. In the experimental test planting in 1941, an increase in the clover leafhopper population was observed during the early season and counts were taken on all of the test varieties during mid-July and again in late August. A total of 25 sweeps was made on each variety in each of the replicates. The counts (table 2) show very little differ-

TABLE 2.—*Clover leafhopper population counts taken on potato varieties in the experimental yellow dwarf field planting at Almond, Wis., on July 17 and Aug. 28, 1941*

Variety	Clover leafhoppers counted in 100 sweeps on—		Variety	Clover leafhoppers counted in 100 sweeps on—	
	July 17	August 28		July 17	August 28
	Number	*Number*		*Number*	*Number*
Green Mountain	292	364	Russet Rural	353	319
Sequoia	269	349	Red Warba	307	312
Triumph	344	384	Katahdin	264	347
Pontiac	296	327	Irish cobbler	261	341
Chippewa	278	321	Rural New Yorker	271	297
Sebago	284	309	Russet Burbank	230	279

ence in population between the varieties under test. It is important to note in this connection that between each variety block, 4 rows of Russet Rural acted as buffers so that in no case were the test rows contiguous. Allen and Rieman (*1*), reporting on insects found on the Russet Rural and Sebago varieties in central Wisconsin in 1941, found little difference in clover leafhopper population on these 2 varieties, which is in accord with the present findings. These 2 late-maturing

varieties which show about the same population of clover leafhoppers in net collections are entirely different in their reaction to the yellow dwarf virus. The low yellow dwarf incidence of such varieties as Russet Burbank, Warba, and Sebago does not seem to be due, therefore, to any avoidance of these varieties by the vector. If resistance is due to escape, as was thought to be possible in an earlier report (*11*), it must be explained on the basis of ineffective transmission of the virus by vectors even though they are present.

<div align="center">GREENHOUSE EXPERIMENTS</div>

In a series of experiments set up under controlled conditions in the greenhouse during the early winter of 1940–41 to study clover leafhopper activity, transmission, and resistance, three late-maturing varieties were compared. Green Mountain and Russet Rural were included because of their known susceptibility to the yellow dwarf virus and Sebago was selected because it had shown a very high degree of resistance under field conditions in the 1939–40 tests. Greenhouse tuber-indexed, disease-free seed was used in the trials which were conducted at a temperature that fluctuated between 24° and 28° C., favorable to both the insect vector and the expression of yellow dwarf symptoms.

In each of 3 transmission tests, 20 viruliferous adult clover leafhoppers were transferred by means of a special sucking pipette to each of 5 caged test plants from different tuber seed pieces of each variety. White sand was used to cover the soil in each caged test pot to facilitate observation of vector activity and to aid in the removal of the vectors after the inoculation period. A like number of caged plants from corresponding tubers with nonviruliferous leafhoppers and without leafhoppers was also included.

During the 14-day feeding period, frequent observations were made to determine the clover leafhopper activity on tne respectiv evarieties as to feeding habits, location of feeding, and longevity on a specific variety. No differences in leafhopper activity on the three varieties under test could be noted and there was no marked decrease in the number of live leafhoppers after the feeding period on any one variety.

At the end of the inoculation period all the viruliferous and nonviruliferous leafhoppers were removed from the cages by the aspirator method and the plants were held for observation. Symptoms of primary yellow dwarf developed on all of the 15 Green Mountain and Russet Rural test plants on which viruliferous leafhoppers were caged; in some plants symptoms were slightly more pronounced than in others. In the Sebago, only 4 plants out of a total of 15 in the entire series developed typical primary yellow dwarf symptoms; 2 plants developed faint symptoms. On 9 plants no recognizable primary symptoms could be seen. In plants on which the nonviruliferous leafhoppers were caged no symptoms were observed. All caged plants without leafhoppers also remained healthy. Tubers produced by the inoculated and uninoculated test plants were stored after harvest. The occurrence of secondary yellow dwarf in the plants arising from these tubers is recorded in table 3. The 15 second-generation Green Mountain and Russet Rural plants all showed typical secondary yellow dwarf symptoms, whereas, as stated above, only 6 inoculated Sebago plants showed typical symptoms. None of the test plants to

which nonviruliferous clover leafhoppers had been added or the non-insect-caged controls developed secondary yellow dwarf symptoms.

The greenhouse experiments confirm in a general way the field findings of Sebago resistance, although the percentage of infected plants was much higher in the greenhouse than in the field. The results show that although the same number of leafhoppers feed actively on each plant, certain Sebago plants are much less likely to be infected than Green Mountain or Russet Rural.

TABLE 3.—*The occurrence of yellow dwarf in the progeny of Green Mountain, Russet Rural, and Sebago plants inoculated with viruliferous clover leaf-hoppers in the greenhouse* [1]

Variety	Number of test plants inoculated	Number in which the progeny showed typical secondary yellow dwarf symptoms	Number in which the progeny showed no yellow dwarf symptoms
Green Mountain	15	15	0
Russet Rural	15	15	0
Sebago	15	16	9

[1] All uninoculated plants remained healthy.

DISCUSSION

The standard potato varieties Russet Burbank, Red Warba, and Sebago tested in an area of central Wisconsin where the yellow dwarf virus commonly builds up rapidly, showed a reasonable constant degree of resistance under field conditions.

The differences in seasonal yellow dwarf infection are governed by environment and influenced directly by the number of insect vectors present. There is, however, no direct correlation between the length of growing season, i. e., time of maturity of a variety, and resistance to the yellow dwarf virus. The two most resistant varieties under test were in one case a very early maturing one (Warba) and in the other a very late maturing one (Sebago).

Net sweepings on the potato varieties under test showed very clearly that the insect vector was equally abundant on all varieties. This in part has been observed by others (*1*). The low incidence of disease in certain varieties after exposure to heavy virus infection in the field is not the result of avoidance of any variety by the vector, as was though to be the case in preliminary studies (*11*), but rather in exclusion of the virus in certain individuals in a clone.

Whereas individuals of a variety, being members of a clone, are essentially buds from a single original plant, inherent difference between individual plants in true resistance can hardly be expected, since some members of the clone once infected are as susceptible as those of any other variety. When Sebago was exposed to viruliferous clover leafhoppers in the green house the percentage of infected plants was higher than in either of the two field tests. This would seem to indicate that the insect vector, even though as abundant on plants of the resistant variety as on those of the susceptible, does not actually cause as many cases of infection on the former in the field, but that the incidence of infection can be increased under certain

conditions in the greenhouse. This does not prove, however, that it is a matter of escape. Rather it shows that the plants escape infection not because the insects avoid them but because the insects are not able to infect them as readily as they do those of susceptible varieties. Whatever resistance may consist of, it is effective at the threshold of infection rather than after the virus has become established.

The low percentage of nonemerging hills in the more resistant varieties (table 1) is of special interest. The "poor stand" phase of the potato yellow dwarf disease under the conditions of relatively high soil temperature that prevail in central Wisconsin has been recognized as an important factor in potato production (*11*). It should be pointed out in this connection that varieties showing a high degree of field resistance, as a rule show also a low percentage of nonemerging hills. Better stands were obtained with resistant varieties exposed to infection than with susceptible varieties. Of even greater significance is the fact that virus-infected tubers of such varieties as Russet Burbank, Warba, and Sebago do not show to any extent the characteristic tuber malformation or the diagnostic internal rusty brown flecking, both associated with yellow dwarf infection, that is commonly found in the more susceptible varieties. This is an important item in relation to table market quality, particularly in the late-maturing varieties.

The planting of a late-maturing variety resistant to the yellow dwarf virus would not only increase yield and quality of table stock produced in the central Wisconsin area but would greatly reduce the cost of production with the elimination of the yearly purchase of seed stocks from yellow-dwarf-free areas.

SUMMARY AND CONCLUSION

The yellow dwarf disease of potato is an important factor in production in certain sections of Wisconsin where the virus commonly builds up rapidly in spite of the increased yearly planting of disease-free seed.

Varieties Russet Burbank, Warba, and Sebago have shown a high degree of field resistance to the yellow dwarf virus under conditions of extensive spread of the virus. This is indicated both by a low percentage of visibly infected hills and of nonemerging hills. These varieties when infected also show little if any tuber malformation or internal necrotic flecking.

In the field, the clover leafhopper, which transmits the disease, was found to be as numerous on resistant as on susceptible varieties.

No difference was observed in clover leafhopper feeding habits or location of feeding on the varieties under greenhouse culture tests. The chances of infecting certain Sebago plants are very much less than those of infecting Green Mountain or Russet Rural.

High incidence of the disease can be most effectively reduced by the use of a vriety or varieties resistant to the virus.

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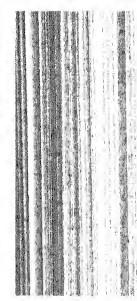
SYSTEMIC INVASION OF CABBAGE SEEDLINGS BY THE DOWNY MILDEW FUNGUS [1]

By F. J. LeBeau [2]

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INTRODUCTION

In southern Mississippi it is a common practice to sow cabbage (*Brassica oleracea* var. *capitata* L.) seed in coldframes in October and early November to produce plants for the early spring crop. Epidemics of cabbage mildew (*Peronospora parasitica* (Fries) Tul. frequently occur and cause heavy losses in such plantings when the seedlings are 2 to 3 weeks old. In the course of investigations into the origin of these epidemics it was found that while the oospores of the mildew organism were rare in the diseased tissue of stems and leaves they did form in the cotyledons (8).[3] Since it is a common practice to grow cabbage plants in seedbeds at close quarters and on the same sites from year to year, it was obvious that over-summering oospores might be the chief source of inoculum in the fall sowing of plant beds. It is the object of this paper to present data on the mode of infection of cabbage seedlings by *P. parasitica* and upon the subsequent development of the disease, particularly with reference to development of epidemics in cabbage plant beds in southern Mississippi. The work was conducted at the Truck Crops Branch Experiment Station at Crystal Springs, Miss.

MATERIAL AND METHODS

Cabbage seedlings were grown from surface-sterilized seed planted in sterile soil either in cotton-stoppered fruit jars or in open cans. The inoculum, consisting of a suspension of conidia, was applied to specific predetermined points on various parts of the plants 5 to 10 days after emergence. That portion of the plant to which the inoculum was applied was kept humid for 24 hours, after which the plants were placed near a south window in the laboratory. Where plants were grown in open cans humidity was low enough to prevent sporulation and thus preclude secondary infection. When stoppered fruit jars were used the experiment was terminated at the occurrence of first sporulation.

For microscopic study the plants were cleared in lactophenol, stained with cotton blue in lactophenol, and mounted whole.

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EXPERIMENTAL RESULTS

INFECTION STUDIES WITH CONIDIA

In each of two trials, five groups of plants were grown in cotton-stoppered fruit jars and kept in darkness for 3 days after emergence, with the result that the hypocotyls were from 5 to 8 cm. long. They were inoculated by placing a drop of a conidial suspension at the base of the hypocotyl, and the first signs of sporulation appeared 6 or 7 days later. The plants were then taken up and segregated with reference to the position of sporulation on the plant. Approximately the same number of plants grown under similar conditions, but not inoculated, remained healthy. The results of these experiments are presented in table 1.

TABLE 1.—*Relation of hypocotyl inoculation to point at which the mildew fungus sporulated after infection*

| Experiment No. | Group No. | Plants inoculated | Total plants sporulating | Plants sporulating on— | | |
				Hypocotyl only	Hypocotyl and cotyledons	Cotyledons only
		Number	*Number*	*Number*	*Number*	*Number*
1	1	23	7	5	2	0
	2	19	4	3	1	0
	3	20	8	4	4	0
	4	15	5	2	3	0
	5	22	7	4	3	0
2	1	12	6	4	2	0
	2	15	8	5	3	0
	3	14	8	7	1	0
	4	10	7	6	1	0
	5	18	13	9	4	0

Representative plants from each group were cleared, stained, and examined microscopically. Of those plants on which no signs of sporulation occurred 25 percent were shown to have been infected by the presence of mycelium in the hypocotyl tissues. Examination of plants bearing sporulation revealed that the mycelium had progressed upward through the hypocotyl, extending into the cotyledons in some cases.

Of the 40 infected plants studied in detail, only the hypocotyl was invaded in 6; the hypocotyl and 1 or both cotyledonary petioles in 6; the hypocotyl, 1 or both cotyledonary petioles, and the basal portion of one or both cotyledons invaded but with no sporulation in 18; all 3 regions were invaded and sporulation occurred on the cotyledons in 10; the lower portion of the hypocotyl had been invaded in all. At a point corresponding approximately to the point of inoculation, the cortical tissue of the hypocotyl was completely invaded by a network of branching hyphae. Upward from this point branching decreased until there remained a comparatively few straight large hyphae which ran intercellularly and parallel to the long axis of the plant. Where branches occurred they usually followed a path parallel to and only a few cells removed from the mother hypha. The hyphae were abundantly supplied with large, well-defined haustoria (figs. 1 and 2). Continuing upward through the cortical tissues of the hypocotyl these hyphae passed through the cotyledonary node into the cotyle-
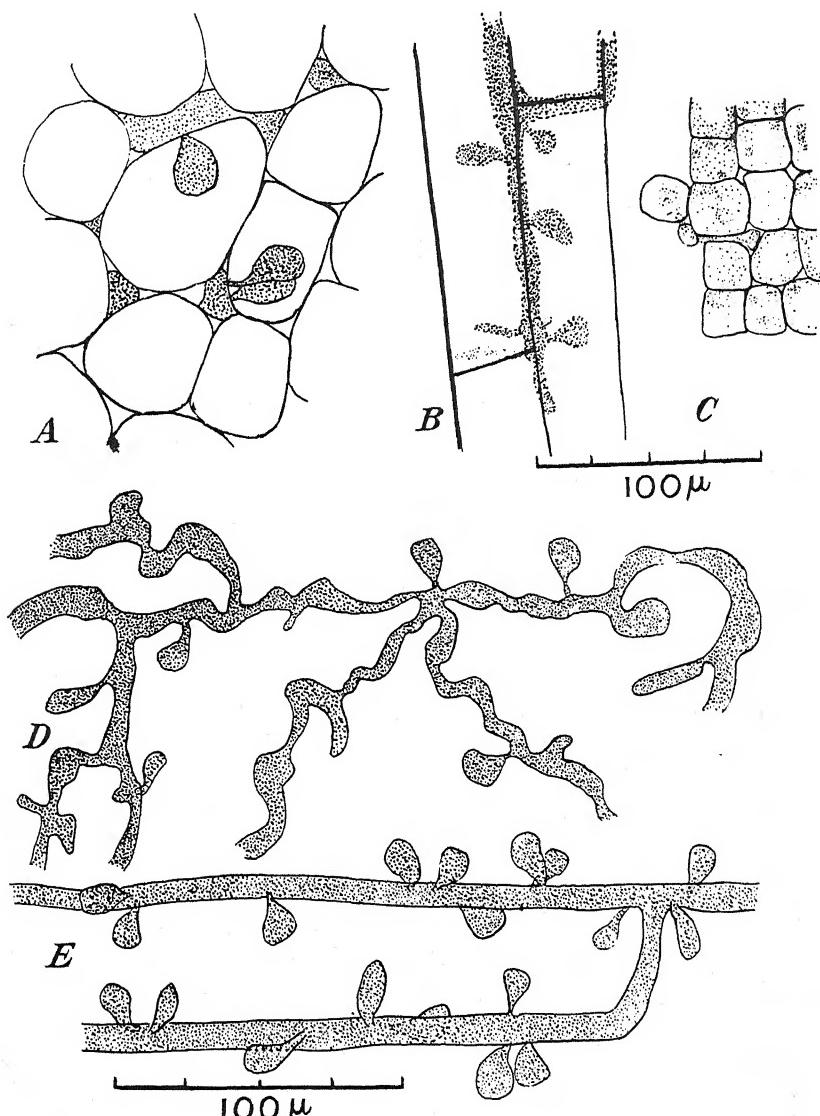
FIGURE 1.—Semidiagrammatic drawings of certain aspects of the pathological histology of cabbage seedlings systemically infected with *Peronospora parasitica* (drawings made with aid of a camera lucida). *A*, Transection of cortical tissues of hypocotyl of a 7-week-old cabbage seedling which survived systemic invasion by *Peronospora parasitica*, illustrating the intercellular mycelium and haustoria. *B*, Longitudinal view illustrating the characteristic branching of the systemic mycelium in the hypocotyl of an infected plant. *C*, Germination of a conidium and penetration of the germ tube between the epidermal cells of the hypocotyl of a cabbage seedling. *D*, Characteristic systemic mycelium as seen in cleared and stained whole mount of a cotyledon of a systemically infected cabbage seedling. *E*, Characteristic systemic mycelium as seen in a cleared and stained whole mount of the hypocotyl of a systemically infected cabbage seedling.
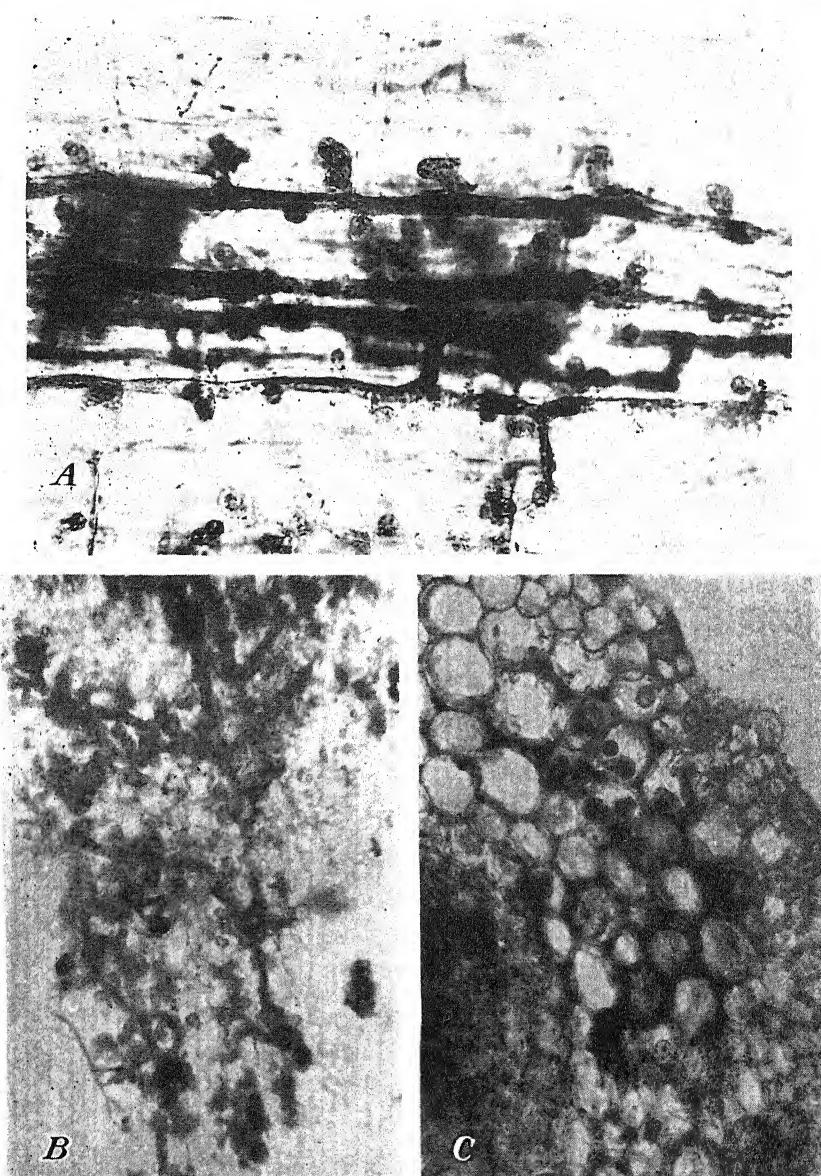
FIGURE 2.—Photomicrographs of cabbage seedlings systemically infected with *Peronospora parasitica*: Cleared and stained whole mount of a hypocotyl (*A*) and freehand tangential section of a cotyledon (*B*), showing characteristic systemic mycelium; transection of the hypocotyl of a 7-week-old seedling (*C*) which survived systemic invasion (note haustoria in the cortical cells).

donary petiole and finally into the lamina of the cotyledon. Tufts of conidiophores appeared at occasional points along the hypocotyl and cotyledonary petiole. In many instances in cleared and stained material single hyphae were traced from the base of the hypocotyl

through the intervening tissues into the cotyledon. At the base of the cotyledonary blade the long straight hyphae lost their identity and the uniform tubular hyphae changed into an irregular, knotty, much-branched mycelium with few well-defined haustoria (figs. 1 and 2). From masses of branching mycelium beneath the stomata conidiophores pushed through the stomata. Except for one questionable case, the mycelium never passed through the cotyledonary node into the next internode of true stem tissue.

In another trial seedlings growing in sterile soil in tin cans were inoculated with a suspension of conidia 5 days after emergence. The plants in each of the five cans were divided roughly into two groups by a piece of cardboard pressed down edgewise into the soil surface. The plants on one side were inoculated by placing a drop of the conidial suspension in a small depression of the soil at the base of the hypocotyl. The soil was then covered with moist absorbent cotton. The remaining plants were similarly treated, sterile water being substituted for the spore suspension. After 24 hours the absorbent cotton was removed and the plants were incubated in the dry atmosphere of the laboratory. Eight days after inoculation two of the five groups of plants were placed in a moist chamber. Five days later the remaining three groups were similarly treated. A record of the sporulation on these five groups of plants is presented in table 2. The controls in all cases remained healthy.

TABLE 2.—*Sporulation on plants inoculated with Peronospora parasitica at base of hypocotyl and placed in moist chambers 8 and 13 days after inoculation*

Group No.	Plants inoculated	Period from inoculation to placement in moist chamber	Plants showing sporulation in—			
			9 days	10 days	11 days	14 days
	Number	*Days*	*Number*	*Number*	*Number*	*Number*
1	7	8	6	0	0	
2	13	8	7	2	2	
3	8	13				6
4	10	13				5
5	11	13				8

After observations on sporulation had been made the plants were cleared, stained, and examined microscopically. In each case of plants on which sporulation developed the hypocotyl was invaded from the base upwards, and the mycelium extended into the cotyledons. These plants presented the same picture of pathological histology as described in the previous experiment. Without exception, whenever the cotyledons were invaded the hypocotyls were also affected, indicating that in all cases infection had occurred at the point of inoculation and not by the chance infection of the cotyledons by conidia. In no case was the mycelium found to have invaded the true stem tissue above the cotyledonary node.

An interesting observation was made on the effects of systemic invasion of the seedling tissues by the mildew fungus. At the time the last three groups were placed in the moist chamber, many of the plants showed collapse of the hypocotyl resembling the signs of damping-off although no other symptoms of mildew prevailed. The

cotyledons were shriveled, necrotic, and desiccated (fig. 3, *A*, *c–h*,). On the other hand, the plants of groups 1 and 2 (fig. 3, *B*, *c–e*) which had been placed in the moist chamber 5 days previously and before signs of injury were apparent, were all turgid. Aside from the mass of sporulation on the affected tissues, they appeared to be vigorous in spite of the disease. The importance of this observation in interpreting certain aspects of disease epidemics in the field will be discussed later.

In a third experiment 12-day-old cabbage seedling growing in sterile soil in tin cans were inoculated at a point approximately 2 cm. above the soil surface. This was accomplished by cutting slits in small corks, which were so placed that they held firmly around the hypocotyl leaving one side exposed. A drop of a suspension of conidia was applied to the exposed portion of the hypocotyl and covered with a bit of moist absorbent cotton. After 24 hours of incubation the cotton and corks were removed. Beginning 2 days after inoculation, 10 plants each day were severed at the soil line, cleared, and stained for microscopic examination. The controls all remained healthy.

As may be seen from the results in table 3, the invading mycelium rapidly progressed upward from the point of inoculation, finally reaching the cotyledons, after traversing an average of approximately 2 cm. of hypocotyl tissue in 5 to 9 days. As in previous experiments, no stem tissue above the cotyledonary node was invaded. The downward invasion of the hypocotyl tissues progressed more slowly and in only four cases did the mycelium reach the soil level, which was about 2 cm. below the point of inoculation. In other experiments in which the plants were taken up by the roots the mycelium was observed to progress as far as the point of origin of the uppermost secondary roots. During the last 3 days of the experiment, as in previous trials, definite signs of injury were apparent although no sporulation occurred since the plants were kept in the dry atmosphere of the laboratory.

The course of invasion of seedlings following infection of the cotyledons was followed in two experiments. In one of these a series of plants, the first true leaf of each of which was inoculated, was included for comparison. In these trials one cotyledon or the first true leaf was inoculated by placing a small drop of inoculum at about the center of the blade. Because of the waxy surface of the tissues a small drop of spore suspension could be made to adhere at the desired spot. A bit of moist absorbent cotton was placed at the base of each plant and a glass vial inverted over each seedling with the mouth pressed into the soil. After a 24-hour incubation period the vials and cotton were removed. The plants remained in the laboratory atmosphere throughout the experiment. No infection occurred in the controls.

When examined microscopically the major portion of each of the inoculated cotyledons was found to be completely invaded by the mildew mycelium. In most cases evidence of infection was apparent at the time the plants were taken up. The cotyledons were shriveled and necrotic while noninoculated cotyledons remained healthy. In some cases the mycelium had progressed from the lamina of the cotyledons into the cotyledonary petiole, and in some cases into the hypocotyl. The maximum extent of the invasion of the hypocotyl from the cotyledon was approximately 10 mm. When the first true
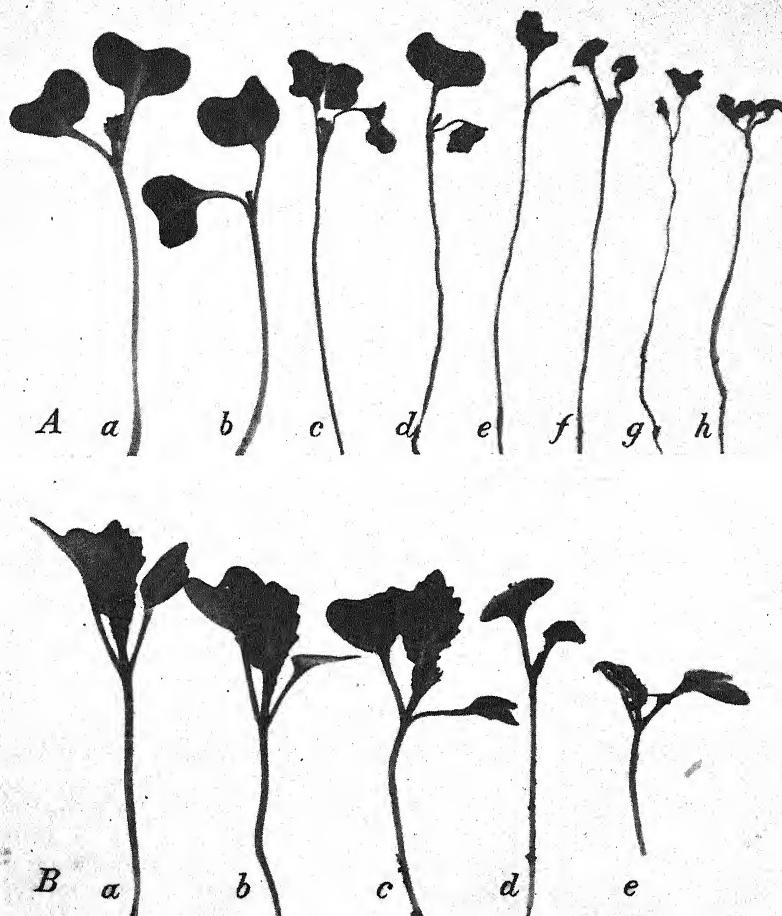
FIGURE 3.—Cabbage seedlings showing the effects of systemic invasion by *Peronospora parasitica*. *A*, Plants kept in a dry atmosphere: *a, b*, Healthy plants; *c, d, e, f, g, h*, infected plants showing different degrees of desiccation of the cotyledons and hypocotyls. *B*, Plants kept in a moist atmosphere: *a, b*, Healthy plants; *c, d, e*, infected plants showing tufts of conidiophores on the cotyledons and hypocotyls.

leaf was inoculated a necrotic spot 3 to 4 mm. in diameter developed at the point of inoculation. Microscopic examination disclosed that an area 3 to 4 mm. in diameter, roughly the area of necrosis, was invaded by the mycelium.

It has frequently been observed that in cabbage seedbeds many seedlings survive a mildew attack even when they have been infected while very young. Surviving plants from such a seedbed sown on November 5, 1942, were selected at the 7-leaf stage, when the hypocotyls were 3 to 5 mm. in diameter, and were sectioned and stained. In 9 of the 28 plants mycelium of the mildew fungus was present in the outer cortical tissue of the hypocotyl, but it had failed to pass

beyond the cotyledonary node into the stem tissue. It appears, therefore, that in plants which survive systemic invasion, the mycelium may remain in the cortical tissues of the hypocotyl for several weeks but does not spread systemically into the stem.

TABLE 3.—*The rate of upward and downward growth of the invading mycelium of Peronospora parasitica in the hypocotyl of cabbage seedlings*

Days after inoculation	Plants studied	Plants infected	Plants in which mycelium reached—		
			Cotyledonary node	Cotyledonary blade	Soil level
	Number	*Number*	*Number*	*Number*	*Number*
2	10	8	0	0	0
3	10	7	0	0	0
4	10	9	2	0	0
5	10	10	3	1	0
6	10	6	5	3	0
7	10	9	7	5	0
8	10	8	8	7	1
9	10	10	10	10	1
10	10	5	5	5	2

INFECTION BY SOIL-BORNE OOSPORES

Soil from a cabbage seedbed in which there had been an abundance of oospores in the cabbage seedling crop of the previous year was placed in each of 12 flats. The soil in 4 flats was disinfected with chloropicrin. Surface-sterilized seed was sown deeply on September 5 and the flats were kept in a coldframe covered with cheesecloth. Pairs of flats were segregated from each other in the coldframe by board walls. Beginning 5 days after emergence, the plants were watered frequently to provide suitable conditions for sporulation. Twelve days after planting the first sporulation appeared. The plants bearing sporulation were removed on each of 3 successive days. Since 3 to 4 days are required from the time of inoculation with conidia for sporulation to occur, it is presumed that few if any of the plants recorded as infected became infected from the conidia produced on the first plant to produce sporulation. The experiment was repeated, beginning on September 24. Infection was obtained in 11 of 16 flats of nonsterile soil, while in only 1 of the 8 flats containing sterile soil did any plants become infected. Examination of infected plants revealed that the mycelium invaded the hypocotyl and progressed systemically in the same manner as described for infection produced artificially. While the results do not furnish complete proof that oospores in the soil were the source of the primary inoculum, they are highly indicative that such was the case. It would appear that in seedbeds in Mississippi initial infection comes from oospores in the soil. Plants thus affected are invaded systemically and when sporulation occurs conidia are provided as secondary inoculum for further dissemination and infection.

DISCUSSION

The question of overwintering or oversummering of *Peronospora parasitica* has been discussed by a number of investigators (*2, 4, 7, 9, 10, 11*), and wild hosts have been widely held to be responsible for the carrying over of inoculum (*2, p. 297–300; 4, 9*). Recent studies on host

specialization in *P. parasitica* (6) have shown that the form which attacks cabbage in this country is peculiar to *Brassica oleracea* and does not attack a wide range of cruciferous species.   Thus the earlier belief that wild hosts are responsible for the carrying over of inoculum of cabbage mildew appears not to be well founded.   That the cabbage mildew organism may overwinter in old cabbage heads and stumps has been pointed out (10, 11).   This means of oversummering is very unlikely in southern Mississippi since cabbage fields are plowed under about 4 months before the seed for the next crop is sown, in which time, by virtue of high temperatures and frequent rains, all traces of old cabbage refuse disappear.   The possibility that summer collards (*B. oleracea* var. *viridis* L.) in home gardens may serve as a means of oversummering of the mildew organism is also questionable.   The collard crop is usually planted in August and therefore is separated from the cabbage crop by at least 2 months.   Further study should be made, however, before collards are completely discounted as a source of inoculum for cabbage mildew.

It appears that oospores serve as the most likely means of over-summering of the cabbage mildew organism.   The occurrence of oospores of the cabbage organism in Europe has been reported (1). The fact that oospores have been found in abundance in the tissues of infected cabbage seedlings (8) and the infection studies herein reported support this belief.

Infection studies with *Peronospora parasitica* have been rather limited.   That the tissues of leaf, cotyledon, hypocotyl, and stem are susceptible to infection by direct penetration of infective hyphae from germinating conidia has been demonstrated (3, 6).   Systemic development of the mildew mycelium in mature cabbage heads in storage (10), in the field (11), and in turnip roots (7) has been reported, but so far as the writer knows, this is the first report of systemic development of *P. parasitica* in the tissues of cabbage seedlings.   The fact that only the hypocotyl and cotyledon appear to be susceptible to this type of invasion is of much interest and appears to be of considerable importance in the epidemiology of the disease.

In southern Mississippi, where cabbage seed for plant production for the early spring crop is sown from October 15 to November 10, severe epidemics of mildew frequently cause a loss of 50 percent or more of young plants.   The extent of the damage varies with the age of the plants when attacked and with weather conditions.   The greatest damage results when plants are infected before the first true leaf is fully developed and when the weather after the appearance of the disease consists of several moist, cool days followed by a prolonged period of dry, sunny weather.   The earlier moist period provides ideal conditions for abundant sporulation and spread of infection from the original centers, as pointed out by Felton and Walker (6), while the dry, sunny period brings about rapid desiccation and necrosis of the infected hypocotyl tissues resulting in death of many seedlings.   If the initial period of abundant sporulation and rapid spread of infection is followed by a prolonged period of quite cool, cloudy weather the survival of plants is considerably greater.   Apparently under such conditions the fungus is retarded by the low temperature, as pointed out by Eddins (5), while the seedlings gradually outgrow the disease.

If the seedlings have reached the 3- to 4-leaf stage when the initial infection appears, the greatest damage results if a prolonged period of

cool, moist weather follows. Under such conditions the damage suffered is primarily one of growth retardation rather than actual killing of the plants. During dry periods such plants rapidly recover from the disease. The ultimate damage is thus determined by the duration of the moist, cool periods. If such periods are of long duration or occur with relatively short intervening dry periods the plants may be set back as much as 3 to 4 weeks. If the dry periods are prolonged, recovery is more rapid and damage is very slight.

The relation of high and low humidities and temperatures on the development of mildew on cabbage plants in the 5- or 6-leaf stage has recently been studied by Felton and Walker (6). In comparing the development of mildew on infected plants which were subjected to continuous high humidity, to alternating periods of high and low humidity, and to continuous low humidity, it was found that the plants under continuous low humidity outgrew the disease most rapidly, especially at the high temperatures. These results under controlled conditions agree with the observed effects under field conditions in Mississippi.

This observed difference between the behavior of mildew on very young seedlings and on older seedlings may be interpreted in the light of the experimental results reported herein. It was shown that in the case of very young seedlings the tissues became thoroughly infected from a single point of inoculation. Therefore, once a general infection was established in the seedbed, the continued production of inoculum diminished in importance. A warm, dry period following a general infection, therefore, did not greatly retard the development of the disease, but rather hastened its course in the infected hypocotyls and made them increasingly susceptible to desiccation during dry, sunny days. That infected tissues rapidly dry out and become necrotic under conditions of low humidity was pointed out by Felton and Walker (6). With older seedlings, in which individual infection points affect only a localized area of the leaf tissues, the ultimate severity of the disease is determined by the sum total of infection points. It is obvious therefore that, to insure a continued severe disease epidemic, conditions favorable for contidued sporulation and infection must be maintained. It has been pointed out that mildew lesions rapidly cease to sporulate when exposed to low humidity (6). Thus continued high humidity is a prerequisite to severe mildew development on these older plants.

The presence of the systemic mycelium in the hypocotyl of plants surviving the mildew attack presents an interesting subject for speculation in view of the reports (10, 11) on systemic invasion of mature cabbage heads by the mildew fungus. Whether a connection can be shown between these two phases of systemic invasion or whether the systemic mycelium in the hypocotl is sloughed off with the cortex as secondary growth proceeds remains to be determined.

SUMMARY

The results of investigations into the epidemiology and parasitism of the mildew fungus (*Peronospora parasitica*) on cabbage seedlings are detailed.

Young cabbage seedlings became systemically invaded by the fungus after infection of the hypocotyl and cotyledonary tissues.

The systemic development of the mildew mycelium was confined to the hypocotyl and the cotyledons, and did not pass through the cotyledonary node to invade the true stem tissues. Invasion of the true leaves remained localized about the point of infection.

Oospores were indicated as the chief source of primary inoculum in the initiation of mildew epidemics in cabbage seedbeds in Mississippi.

The relation of these results to the interpretation of the development of epidemics of cabbage mildew in seedbeds in Mississippi is discussed.

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TRANSPIRATION RESPONSES OF PERSIAN WALNUTS AND FILBERTS SPRAYED WITH BORDEAUX MIXTURE [1]

By P. W. MILLER, *plant pathologist,* and C. E. SCHUSTER, *horticulturist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture*

INTRODUCTION

The widespread use of bordeaux mixture for the control of many plant diseases has made it the subject of numerous investigations. Included among these are studies of its effect on the physiological behavior of the plant. Most of the earlier work on this subject has been adequately reviewed by Duggar and Bonns (*1*),[2] Miller (*4*), and Foster and Tatman (*2*).

The results of previous investigations are conflicting. A number of workers reported finding that the rate of transpiration increased after the application of bordeaux mixture to potted plants or detached leaves. Other workers, however, have found that bordeaux mixture decreases the rate of transpiration or has little or no effect. Still others have found that bordeaux mixture has a retarding effect on photosynthesis and respiration, but little or none on transpiration.

As bordeaux mixture has recently been found to be effective for the control of walnut blight (*5*), caused by *Xanthomonas juglandis* (Pierce) Dowson, and the bacterial blight of filbert (*7*), caused by *Xanthomonas corylina* P. W. Miller et al., it was deemed advisable to ascertain its effect on the transpiration of Persian walnuts (*Juglans regia* L.) and filberts (*Corylus avellana* L.).

MATERIALS AND METHODS

The experiments reported herein were conducted in the greenhouse at Oregon State College, Corvallis, Oreg., from 1939 to 1941, inclusive. Seedling Persian walnuts, the seed of which came from grafted trees of the Franquette variety, and seedling filberts from trees of the Barcelona variety were grown in cans. Coarse gravel to a depth of several inches was placed in the bottom of each of a number of watertight, lacquered, 1-gallon tin cans. An enriched soil composed of approximately equal parts of sand, well-rotted manure, and loam was put above the gravel. One seed of walnut or filbert, previously stratified in sand, was then planted in each can. When the seedling was sufficiently large for use, a rubber boot was placed about the base of the stem to prevent scalding of the tissues and the surface of the soil was covered with a mixture of melted paraffin and vaseline to reduce surface evaporation to a minimum. The soil was kept at the optimum moisture content by the addition of measured amounts of aerated tap water through a piece of glass tubing extending to the bottom of the

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can. Loss of water through the tube was prevented by placing a small piece of wax on its mouth. By means of this device loss of moisture was reduced without the production of anaerobic conditions in the soil, as the entrance of fresh air in small quantities was possible around the stem, between the side walls of the can and the paraffin cover, which invariably pulled away from the can, and in the aerated water. Air was also present in the spaces between the gravel in the bottom of the can, as was evidenced by its forcible expulsion through the glass tube when water was added. The addition of the fresh, aerated water at frequent intervals resulted in a repeated interchange of air, which prevented the accumulation of carbon dioxide and the consequent development of anaerobic conditions.

The plants were paired on the basis of similar leaf areas as determined by tracing the margins of the leaves on paper and measuring them with a planimeter. All except the two basal leaves on each plant were removed. This is permissible since the lower leaves of both walnuts and filberts are structurally the same as the younger, terminal leaves; the only difference is that the old leaves are less active. After the plants were paired, the daily transpiration losses were obtained for each plant for a period ranging from 5 to 10 days to ascertain their pre-spray transpiration ratios. Light and heat conditions in the greenhouse during the experimental period were maintained in as nearly normal condition as possible. The individual plants were moved about systematically on the benches every day to minimize the effect of any possible variations in environment which might occur because of the positions occupied by the plants. Such variations as may have occurred were further minimized by repetitions of the experiments at different times during the growing season and over a period of years. After their pre-spray transpiration ratios had been established the upper and lower bottom surfaces of the leaves of one member of each pair were thoroughly sprayed with a hand sprayer. The formulas of bordeaux mixture used were 6–2–100, 6–6–100, 8–2–100, and 8–8–100; the 6–6–100 formula was used in about three-fourths of the experiments with walnut and the 8–8–100 formula in about half of those with filbert. Daily transpiration losses for the sprayed plant and for the corresponding untreated plant of the pair were then obtained for a subsequent period ranging from 5 to 21 days, and the ratio of the transpiration of the sprayed plant to that of the unsprayed was determined.

TRANSPIRATION RESPONSES TO BORDEAUX MIXTURE

The rate of transpiration after spraying with bordeaux mixture increased in 41 percent of the tests conducted with walnuts, totaling 124 pairs of plants, and decreased in 59 percent. In 54 percent of the tests conducted with filberts, totaling 59 pairs, transpiration increased and in 46 percent it decreased after the leaves were sprayed with bordeaux mixture. Representative results of the experiments are given in table 1. It is evident from an inspection of the data that the results are conflicting. The extent of the increase or decrease in the transpiration rate varied greatly in the different tests. It is worthy of note that, in those cases where the transpiration of the sprayed plants increased after they were sprayed, the acceleration occurred

TABLE 1.—*Representative transpiration ratios of Persian walnuts and filberts before and after being sprayed with bordeaux mixture, 1939–41*

Plant, bordeaux treatment, and pair No.[1]	Pre-spray ratios (to be sprayed: to remain unsprayed)	Post-spray ratios (sprayed: unsprayed)	Difference (pre-spray —post-spray ratio)[2]	Plant, bordeaux treatment, and pair No.[1]	Pre-spray ratios (to be sprayed: to remain unsprayed)	Post-spray ratios (sprayed: unsprayed)	Difference (pre-spray —post-spray ratio)[2]
Persian walnut:				Filbert—Con.			
6–2–100:				6–6–100:			
1	1.02	0.92	−0.10	31	0.94	1.05	+0.11
2	1.01	.98	−.03	32	1.51	1.32	−.19
3	.50	.41	−.09	33	.93	.90	−.03
4	1.52	1.62	+.10	34	.71	1.04	+.33
5	.73	.80	+.07	35	1.25	.70	−.55
6	.60	.60	.00	36	.93	1.13	+.20
7	.72	.70	−.02	8–2–100:			
8	.94	.70	−.24	37	1.32	1.61	+.29
9	.83	.95	+.12	38	1.62	1.52	−.10
10	.47	.38	−.09	39	1.75	.90	−.85
6–6–100:				40	1.32	1.10	−.22
11	1.04	.74	−.30	41	1.26	1.03	−.23
12	1.19	1.15	−.04	42	1.46	1.49	+.03
13	1.14	1.35	+.21	43	1.40	1.23	−.17
14	.95	.82	−.13	44	1.05	.90	−.15
15	1.23	1.26	+.03	45	1.39	1.13	−.26
16	1.00	1.80	+.80	46	.85	1.11	+.26
17	1.03	.63	−.40	8–8–100:			
18	.80	.93	+.13	47	1.30	1.35	+.05
19	.82	1.04	+.22	48	1.13	1.05	−.08
20	1.01	.97	−.04	49	.90	.95	+.05
Filbert:				50	.47	.67	+.20
6–2–100:				51	1.09	.95	−.14
21	1.00	1.14	+.14	52	.61	.55	−.06
22	.81	.67	−.14	53	.92	1.06	+.14
23	.91	1.18	+.27	54	.96	.90	−.06
24	.75	.59	−.16	55	.47	.35	−.12
25	.95	1.71	+.76	56	.99	1.21	+.22
26	.96	1.03	+.07	57	.81	.81	.00
27	.96	.90	−.06				
28	.60	.64	+.04				
29	1.01	1.19	+.18				
30	1.29	1.51	+.22				

[1] Paired on the basis of similar leaf areas.
[2] Plus sign indicates an increase in transpiration; minus sign, a decrease.

immediately after spraying and then the transpiration of the sprayed plants decreased rapidly until the plant was transpiring at or close to the pre-spray rate.

From a hasty survey, it might be concluded that the wide variability in the data given in table 1 was due to poorly matched test plants. However, as previously stated (p. 466), the plants were first paired on the basis of leaf area, a basis for pairing used by a number of investigators (*2, 4*). The transpiration responses of the paired plants for a 5- to 10-day period before spraying were then ascertained. Strange as it may seem, the transpiration rate of two plants having practically the same leaf area varied widely in many cases; this explains why the pre-spray ratios, as recorded in column 2 of table 1, varied so much.

The results of studies carried on by Wilson and Runnels (*8*), Horsfall and Suit (*3*), and others indicated that the higher the proportion of lime in bordeaux mixture the greater the increase in the transpiration rate. Wilson and Runnels (*8*) reported that a 6–6–100 bordeaux mixture increased the transpiration rate of sprayed coleus plants about twice as much as did a 24–6–100 bordeaux mixture.

Investigations carried on by the senior author (*6*) have shown that a highly alkaline bordeaux mixture causes considerably more damage to walnut foliage than does a low-lime or neutral bordeaux mixture,

It was, therefore, deemed advisable to determine the comparative effects of low- and high-lime bordeaux mixtures on the transpiration rates of walnuts and filberts. In table 2 are summarized the results of pertinent experiments.

TABLE 2.—*Comparison of transpiration responses of Persian walnuts and filberts to spraying with low- and high-lime bordeaux mixtures, 1939–41*

Plant and bordeaux formula	Number of pairs	Transpiration rate after spraying	
		Increased	Decreased
		Percent	*Percent*
Persian walnut:			
6–2–100	11	63.6	36.4
6–6–100	6	50.0	50.0
Filbert:	18	44.4	55.6
6–2–100	23	47.8	52.2
6–6–100			
8–2–100	21	47.6	52.4
8–8–100	62	50.0	50.0

It is evident from table 2 that the transpiration responses of both Persian walnuts and filberts after the application of a highly alkaline bordeaux mixture were not markedly different from those observed after the application of a low-lime bordeaux mixture. This may possibly be due to the use of mature leaves in these experiments. It is a known fact that in nature old leaves are much less active physiologically than younger ones.

DISCUSSION

The most distinctive feature of the data reported herein is their great variability. Just why the transpiration rate should increase after the application of bordeaux mixture in one experiment and decrease after its application in another carried on under the same or similar conditions is difficult to understand. It would normally be expected that within certain limits the plants of the same age and vigor, grown under the same environmental conditions, would respond similarly to any given treatment. In certain instances there were slight differences in the age of the leaves of the test plants, though the consistent use of the basal leaves in all tests should have minimized the chance of the differences in the activity level being mainly responsible for the variability of the results. Some of the variability could conceivably be due to differences in the number of stomata per leaf, though counts of them on representative leaves of comparable ages on different plants showed no marked differences in the number present. Since the plants used were seedlings, it is possible that the variability of the results may have been due to the existence of inherent, biological differences in the individual plants. There is the added possibility that the inconsistencies may be associated with the prevalence of structural or physiological differences between different groups of plants by virtue of differences in the fertility of various soil mixtures used in the different seasons.

The lack of a consistent response to applications of bordeaux mixture may possibly be associated with the use of older leaves in these experiments. It is quite possible that these mature leaves did not respond so readily to bordeaux mixture as younger, more active leaves might have done.

SUMMARY

The results of investigations on the transpiration response of Persian walnuts and filberts to spraying with bordeaux mixture are reported.

Under the conditions of these experiments no uniform effects on the transpiration rate of either Persian walnuts or filberts followed the application of bordeaux mixture. The response of both walnuts and filberts after being sprayed with a high-lime bordeaux mixture were not markedly different from those of plants sprayed with a low-lime bordeaux mixture.

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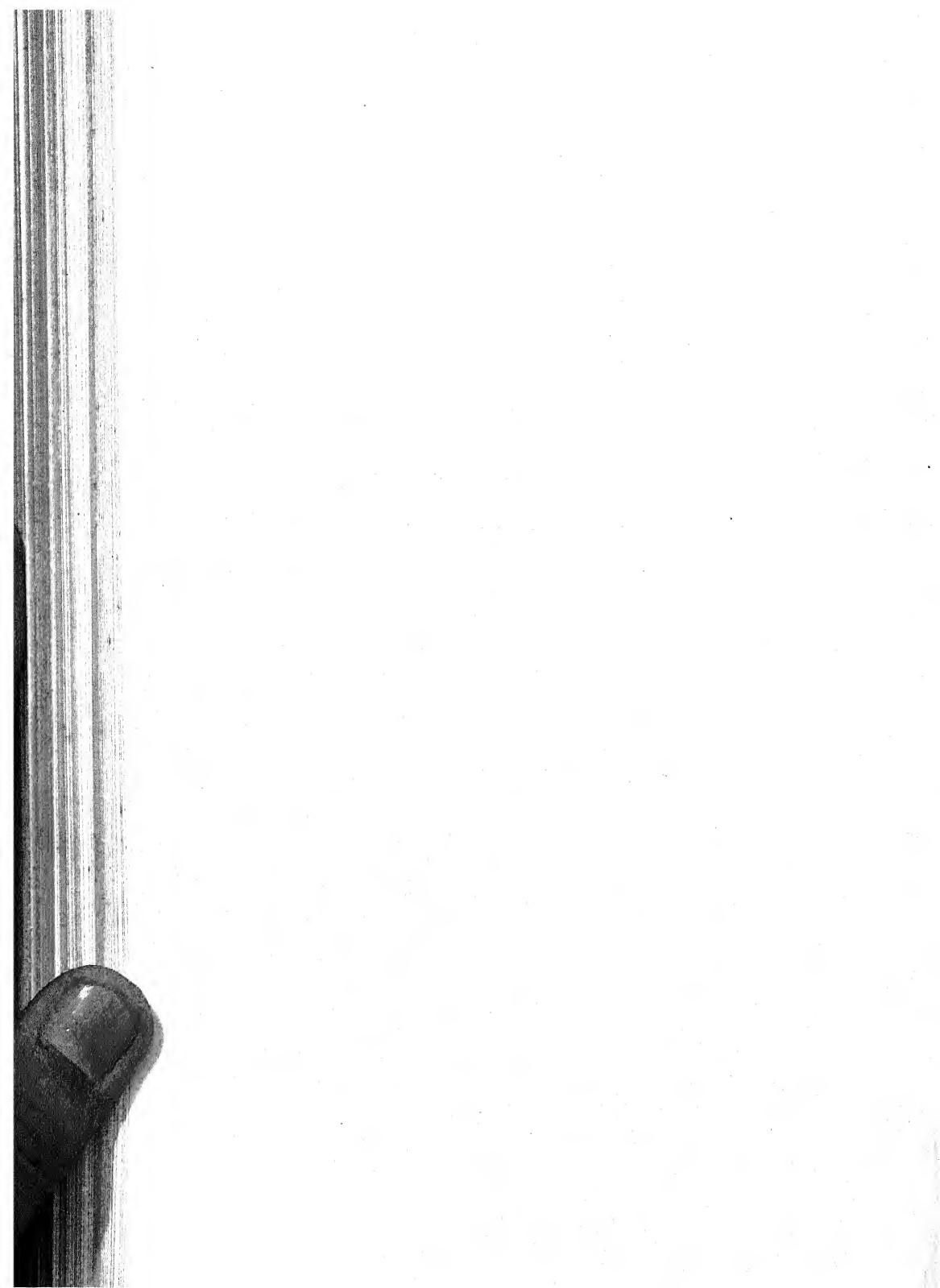
EFFECT OF AIR TEMPERATURE ON THE CONCENTRATION OF CERTAIN VIRUSES IN CABBAGE[1]

By GLENN S. POUND, formerly *research assistant*, and J. C. WALKER, *professor of plant pathology, Wisconsin Agricultural Experiment Station* [2]

INTRODUCTION

The crucifer viruses appear to belong to two distinct groups (*5*).[3] One group, represented by *turnip virus* 1 Hoggan and Johnson, contains cabbage virus A (*12*), the cabbage black ring virus (*10*), the cabbage ring necrosis virus (*3*), Tompkins' turnip virus (*9*), Hoggan and Johnson's turnip virus (*2*), turnip viruses described by LeBeau and Walker (*4*), and probably others (*5*). The second group, represented by *cauliflower virus* 1 Tompkins, contains cabbage virus B (*12*), the cauliflower mosaic virus (*8*), Tompkins' Chinese cabbage virus (*11*), and the broccoli virus of Caldwell and Prentice (*1*). Viruses belonging to the first group are favored by relatively high air temperatures, those of the second group by relatively low air temperatures (*5*). Symptoms produced by members of each group tend to become masked at unfavorable temperatures. When a virus of one group occurs in the host plant together with a virus of the other group, the resulting disease reaction is more severe than that of either virus alone, especially at high temperatures.

The present paper is a report of studies on the effect of air temperature and day length on the concentration of cabbage virus A, a member of the turnip virus group, when it occurs alone in cabbage and when it is accompanied by cabbage virus B, a member of the cauliflower virus group. The relation of environmental factors to seasonal fluctuation of symptoms of cabbage mosaic and the interaction of viruses A and B when they occur together in cabbage was also investigated. The black ring virus and the cauliflower mosaic virus were included in one experiment as a further test of their relationship to virus A and virus B, respectively.

MATERIALS AND METHODS

The Jersey Queen variety of cabbage (*Brassica oleracea* var. *capitata* L.) was used throughout the greenhouse experiments. Plants were inoculated in the 3- or 4-leaf stage by methods described previously (*5*). All plants which failed to develop symptoms after a given period were discarded. Plants were grown in 4-inch clay pots in greenhouse soil which was turned and sifted a number of times before being used in order to obtain as great a degree of uniformity as possible. Special effort was made to keep the soil moisture content

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of all pots the same. Uniformity of light conditions was provided by growing the plants directly beneath fluorescent lights in the greenhouse. Temperatures of 16° and 28° C. were maintained in the greenhouse studies, and all plants were kept at a constant temperature of 22° before they were moved to these temperatures for inoculation. In setting up an experiment three times the number of plants needed were potted in order that plants could be closely culled for uniformity at the time of inoculation.

In preparing the inoculum to be tested for virus content [4] small disks of tissue, all of equal area, were cut from the youngest available leaves of the cabbage plants and macerated in a given amount of distilled water until the mixture was completely homogeneous. The usual procedure was to take 1 tissue disk from each of 10 plants and macerate the combined samples in 10 cc. of water. In any comparison an equal number of tissue disks was taken from the same number of plants at each temperature, care being taken that the leaves used were exactly comparable.

The number of local lesions (fig. 1) produced on inoculated leaves of tobacco plants (*Nicotiana tabacum* L. var. Connecticut Havana 38) was used as a measure of the virus concentration in samples of juice extracts. Spencer and Price (7) and Price and Spencer (6) have pointed out that the local-lesion method merely measures virus activity. Thus only when two virus preparations differ only in the concentration of the infectious entities do local-lesion counts become a measure of differences in virus concentration. It was considered that in these studies the differences in lesion counts at high and low temperatures represented differences in virus concentration since temperature was the only variable involved. The half-leaf method of comparison was used in all experiments carried out under controlled greenhouse conditions. Whole-leaf comparisons were used for inoculations from field-grown plants. Finely powdered carborundum was sprinkled as evenly as possible on both halves of leaves before inoculation. Although virus A will produce a limited number of lesions without carborundum, preliminary trials indicated that the infectivity of the virus was so low that greater variation between halves of the same leaves occurred when carborundum was not used than when it was used. Uniformity tests described in the following section show that the use of carborundum did not introduce any significant variation. All plants were sprinkled with carborundum before any leaf was designated to receive a particular treatment. Any particular treatment was placed an equal number of times on the right and left halves of leaves. Plants used in any one experiment were all of the same age and were carefully chosen for uniformity. Before inoculation the growing point and all excess leaves were removed from the plants. Inocula were applied with glass spatulas.

EXPERIMENTAL RESULTS

INOCULATIONS FROM FIELD PLANTS

Since it had been observed that symptoms of virus A diminished as the fall weather became cooler, it was thought that the concentration of this virus in plants might also diminish as the temperature became lower. Therefore, in the summer of 1941, 27 plants were chosen from

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[4] The term "virus content" used in this paper refers only to the virus activity of expressed sap as measured by local-lesion counts on *Nicotiana tabacum*.
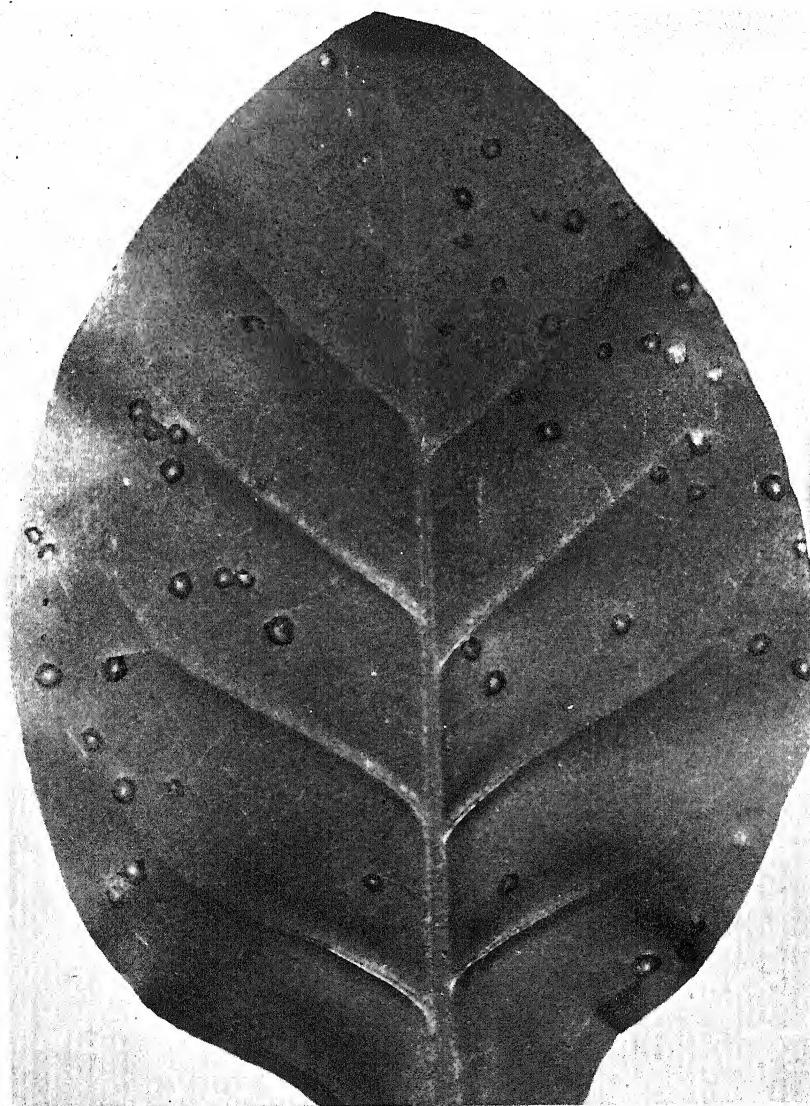
FIGURE 1.—Local lesions produced on inoculated leaves of *Nicotiana tabacum* by cabbage virus A. The black-ring virus produces an identical reaction on this host.

a cabbage mosaic field plot as showing typical symptoms of the A+B virus complex. Inoculations to cabbage and tobacco were made on August 23, September 12, October 14, and November 6 from these plants while they were still in the field. During this time the daily mean temperature gradually became lower and the plants withstood a few light freezes. The average mean daily temperature for August, September, October, and November was 22.2°, 18.2°, 11.8°, and 4.2° C., respectively. On November 11 the plants were dug up and placed in pots in a coldframe where the temperature was maintained at about 5°. A fifth inoculation was made on January 29, or some 9 weeks after plants were removed from the field. On that date the plants were transferred to a greenhouse temperature of 24°. A final inoculation was made on February 16. In making the inoculations the youngest leaf tissue available was macerated in a sterile mortar and filtered through several layers of cheesecloth. Equal quantities of the undiluted extract were used to inoculate both cabbage and tobacco plants. Before the plants were mature in the field, inoculum was taken from the inner head leaves. After heads were cut, young leaves from new growth at the leaf scars were used.

In table 1 are given the results of the inoculations from 14 plants. The remaining 13 plants did not survive the long period in the cold-frame. The data show an apparent decrease in concentration of virus A as the temperature decreased and an increase in concentration when plants were exposed again to high temperatures. This is indicated by the reaction on both cabbage and tobacco. It is of interest to note that tobacco was infected at lower concentrations than cabbage.

This experiment was repeated in 1942 but unfortunately only three inoculations were made from the plants. A severe freeze in mid-October terminated the experiment. A study of table 2, however, will show that results similar to those of 1941 were obtained.

TABLE 1.—*Concentration of cabbage virus A as measured at frequent intervals in cabbage plants infected with viruses A and B and exposed to decreasing temperatures until January 29 when the temperature was increased to 24° C.; 1941*

Plant No.	Number of lesions on 6 tobacco leaves inoculated [1]—						Type of symptoms produced on cabbage inoculated [2]—					
	Aug. 23	Sept. 12	Oct. 14	Nov. 6	Jan. 29	Feb. 16	Aug. 23	Sept. 12	Oct. 14	Nov. 6	Jan. 29	Feb. 16
1	198	+	0	0	1	68	A+B	B	—	B	A+B	A+B
2	315	+	47	3	0	277	A+B	B	B	B	—	A+B
3	140	+	32	0	0	98	A+B	B	B	B	—	A+B
4	287	+	41	0	2	73	A+B	—	—	B	B	A+B
5	127	+	0	2	1	9	A+B	A+B	B	B	—	A+B
6	162	+	29	2	2	43	A+B	—	B	B	B	A+B
7	211	+	0	5	0	301	A+B	A+B	B	B	B	A+B
8	114	+	19	0	5	81	A+B	—	B	B	B	A+B
9	87	+	21	50	0	34	A+B	B	B	B	B	A+B
10	182	+	0	4	5	57	A+B	B	—	B	B	A+B
11	106	+	42	3	1	16	A+B	—	B	B	B	A+B
12	101	+	0	0	0	36	A+B	—	—	B	B	A+B
13	254	+	139	201	0	68	A+B	A+B	A+B	A+B	B	A+B
14	119	+	17	0	0	49	A+B	B	B	B	B	A+B

[1] + indicates positive reaction; − indicates negative reaction; the number of local lesions were not counted in the inoculation of Sept. 12.

[2] A+B indicates symptoms were those of plants known to contain both virus A and B; B indicates symptoms were those of plants known to contain only virus B; − indicates no symptoms.

TABLE 2.—*Concentration of cabbage virus A as measured at frequent intervals in cabbage plants infected with viruses A and B and exposed to decreasing temperatures; 1942*

Plant No.	Number of lesions on 8 tobacco leaves inoculated—			Symptom type on cabbage inoculated [1]—		
	Aug. 12	Sept. 11	Oct. 10	Aug. 12	Sept. 11	Oct. 10
1	258	8	0	A+B	—	B
2	121	5	0	A+B	B	B
3	88	58	4	A+B	A+B	B
4	112	20	4	A+B	B	B
5	435	118	3	A+B	A+B	B
6	184	161	3	A+B	A+B	B
7	237	109	11	A+B	A+B	B
8	67	38	0	A+B	B	B
9	531	180	0	A+B	A+B	B
10	50	110	0	A+B	A+B	B
11	301	261	4	A+B	A+B	B
12	62	36	0	A+B	B	B
13	34	78	0	A+B	B	B
14	110	11	2	A+B	B	B
15	208	180	0	A+B	A+B	B

[1] A+B indicates symptoms characteristic of plants containing both viruses A and B; B indicates symptoms characteristic of plants containing only virus B; — indicates no symptoms.

In 1942 an experimental planting of kale (*Brassica oleracea* L. var. *acephala* DC.) became heavily infected with cabbage virus A. In late season these plants were removed from the field and placed in a coldframe where the temperature was maintained at about 5° C. On February 3, 1943, after some 3 months at this low temperature, a test inoculation to tobacco was made from 10 such plants, 8 tobacco leaves being used in each test. The kale plants were then transferred to a greenhouse temperature of 24°, and a second inoculation was made on February 23 in exactly the same manner as the earlier inoculation. The results are shown in table 3.

In these experiments involving field plants many uncontrolled variables existed, and if the differences obtained were not so great little significance could be attached to them. In making periodic inoculations from growing plants each inoculum was not exactly comparable to the preceding one. Furthermore, it was necessary to use different tobacco plants for each inoculation, and subsequent data will show that considerable variation exists between tobacco plants in regard to their susceptibility to virus A. In order to circumvent these variables, greenhouse studies were performed in which a similar approach to the problem was made.

TABLE 3.—*Effect of air temperature on the concentration of cabbage virus A in kale plants*

Plant No.	Number of lesions on 8 tobacco leaves—		Plant No.	Number of lesions on 8 tobacco leaves—	
	Feb. 3, 1943 [1]	Feb. 23, 1943 [2]		Feb. 3, 1943 [1]	Feb. 23, 1943 [2]
1	0	282	7	1	879
2	0	497	8	0	382
3	0	0	9	1	640
4	0	414	10	0	519
5	3	1,011			
6	0	364	Total	5	4,988

[1] Inoculation made after plants had grown at 5° C. for 3 months.
[2] Inoculation made 20 days after plants were transferred from 5° to 24°. Four tissue disks were macerated in 4 cc. of distilled water for each inoculation.

GREENHOUSE STUDIES

Uniformity Tests

In making inoculations to tobacco it was necessary to sprinkle powdered carborundum on the leaves in order to get a usable number of lesions, especially if older plants were used. It became necessary then to run uniformity tests in order to ascertain if any significant variation existed between halves of the same leaf when the carborundum method was used. In such trials both halves of all leaves were sprinkled evenly with carborundum, and the same inoculum was applied to all half leaves. Trials were made with both a Latin square and an incomplete block design. The incomplete block design and method of analysis were essentially those described by Youden (*13*) and Youden and Beale (*14*).

In the Latin square design four hypothetical half-leaf comparisons (A, B, C, D) were tested on four plants containing four leaves each according to the following arrangement:

$$
\begin{array}{cccc}
A & B & C & D \\
B & C & D & A \\
C & D & A & B \\
D & A & B & C
\end{array}
$$

In this design each comparison occurred once at each leaf position and once on each plant when the rows are considered as leaves and the columns as plants. This design was especially applicable for virus treatment comparisons, the only requirement being that there be as many leaves on a plant and as many plants as there were treatment comparisons. Unless tobacco plants were grown in large pots the number of available leaves was usually limited to four or five.

By the use of the incomplete block design, uniformity trials were run in which 13 hypothetical half-leaf comparisons (A, B, C, D, E, F, G, H, I, J, K, L, M) were made on 26 plants of 3 leaves each. Each comparison occurred twice at each leaf position and once on the same plant with every other comparison according to the following arrangement:

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A A B B C C D D E E F F G G H H I I J J K K L L M M
B D D E H E F G F L A K J C G B K H A I M L C M I J
C E I M D J J M H G G B B I K L E A K L C D F A F H
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This design proved especially efficient, since a large number of half-leaf comparisons could be made and at the same time leaf position and plant effects could be measured. It was more applicable than the Latin square in that a much greater number of treatment comparisons could be made. Two such uniformity trials were made. Analysis of variance of the data showed that the variation between halves of the same leaves with the carborundum method fell well within the range of experimental error. No significant difference existed between half-leaf comparisons. A marked gradient of susceptibility existed between leaf positions, the uppermost leaves being least susceptible and the lowest leaves being most susceptible. The middle leaves were intermediate in susceptibility. Although fluctuations occurred, this gradient of susceptibility was consistent and occurred throughout the experiments. A marked variation also occurred between individual plants. Results of uniformity tests in which a Latin square was used were similar to those with the incom-

plete-block design as regards variation between halves of the same leaves, between leaf positions, and between plants.   Since these data are being presented elsewhere the detailed lesion counts are not given here.

Temperature and Photoperiod Relations

Since the uniformity trials indicated that the half-leaf method with carborundum would yield reliable comparisons, experiments were performed in which the concentration of virus A was measured in cabbage plants infected with virus A alone and with viruses A and B together and grown under 15-hour (long day) and 6-hour (short day) days at at both 16° and 28° C.   Day lengths were controlled by covering the plants with light-proof cages when the respective day length terminated.   Air ventilation was provided so that the air temperature inside and outside the cage was the same.   Thus with the 6 different treatments 12 half-leaf comparisons were made as follows:

Day length:
    Virus A (long day) at 28° vs. virus A (short day) at 28°.
    Virus A (long day) at 16° vs. virus A (short day) at 16°.
    Virus A+B (long day) at 28° vs. virus A+B (short day) at 28°.
    Virus A+B (long day) at 16° vs. virus A+B (short day) at 16°.
Temperature:
    Virus A (long day) at 28° vs. virus A (long day) at 16°.
    Virus A (short day) at 28° vs. virus A (short day) at 16°.
    Virus A+B (long day) at 28° vs. virus A+B (long day) at 16°.
    Virus A+B (short day) at 28° vs. virus A+B (short day) at 16°.
A vs. A+B:
    Virus A (long day) at 28° vs. virus A+B (long day) at 28°.
    Virus A (short day) at 28° vs. virus A+B (short day) at 28°.
    Virus A (long day) at 16° vs. virus A+B (long day) at 16°.
    Virus A (short day) at 16° vs. virus A+B (short day) at 16°.

One of the treatment comparisons was duplicated each time in order that the same incomplete block design could be used that was used in the uniformity trials   With these comparisons the effect of both day length and temperature on the activity of virus A as well as the effect of virus B on the activity of virus A was measured. The day-length effect was studied since it had been observed in nature that the effect of virus A was at a maximum during warm, long days and declined as plants grew into cool, short days.   It was not certain whether the reduced activity was due to reduced temperature, to reduced day length, or to a combination of low temperatures and short days   It was of interest to measure the effect of virus B on the activity of virus A since it had previously been shown that the effect of these two viruses together was much more severe than that of either virus alone (5)   Since this increased severity was more pronounced at high temperatures where virus A was apparently more active, it became a question as to whether virus B might not enhance the activity of virus A.

In table 4 are given the results obtained in three separate experiments of this nature   In each experiment virus A occurred in much greater amount in cabbage plants grown at 28° C. than in those grown at 16°   The *F* value in each temperature comparison was greater than that at the 1-percent level of probability except in one trial in experiment 1 where it exceeded the 5-percent level of probability.

No significant difference was found between the virus concentrations in plants growing at different day lengths   In all long- and short-day comparisons the F values obtained were without exception smaller than that at the 5-percent level of probability, indicating that the day lengths studied did not affect the concentration of virus A.

TABLE 4.—*Effect of temperature and length of day on the concentration of cabbage virus A in cabbage plants infected with virus A alone and with viruses A and B together in the greenhouse under controlled conditions*

Half-leaf comparisons with virus—	Temperature (°C.)	Length of day [1]	Experiment 1		Experiment 2		Experiment 3	
			Comparison totals	F value [3]	Comparison totals	F [2] value [3]	Comparison totals	F value [3]
A	28	Long day	[2] 304	0.37	512	0.46	227	0.28
		Short day	342		463		205	
	16	Long	178	.39	142	.76	94	.59
		Short	217		205		126	
A+B	28	Long	352	.00	456	2.84	203	1.11
		Short	353		578		247	
	16	Long	128	.08	179	.11	150	.23
		Short	146		155		170	
A	28 16	Long	379 179	10.14**	404 172	10.26**	321 146	17.54**
	28 16	Short	366 187	8.12**	500 180	19.52**	323 150	17.14**
A+B	28 16	Long	272 96	7.85**	387 124	13.18**	218 89	9.53**
	28 16	Short	264 107	6.25*	624 198	34.59**	318 89	30.03**
A / A+B	28	Long	435 372	1.00	421 499	1.16	279 248	.55
A / A+B	28	Short	498 414	1.79	519 657	3.63	254 267	.10
A / A+B	16	Long	212 191	.11	211 175	.25	146 135	.07
A / A+B	16	Short	192 153	.39	198 175	.10	175 131	1.11

[1] Long day and short day refer to 15-hour and 8-hour day, respectively.
[2] Values given are local-lesion totals in 6 replicates.
[3] **Highly significant; *significant; unstarred values are insignificant.

The concentration of virus A in plants infected with both A and B viruses exactly paralleled that in plants infected with virus A alone. In these comparisons no significant differences were found, either under long or short day, or at high or low temperatures. Furthermore, in the comparisons of virus A concentration at the two temperatures, the differences were similar in plants inoculated with viruses A and B and those with virus A only. This would indicate that virus A is quite independent of virus B within the host plant and that this independence is not altered by such environmental factors as temperature and day length. The increased severity resulting when viruses A and B occur together is apparently due to the additive effect of each virus on the host metabolism and does not result from an enhanced activity of virus A.

Although the length of day used did not affect the concentration of virus A, there was a noticeable effect upon host growth and upon the severity of virus symptoms. Under short day at both high and low temperatures healthy cabbage plants were chlorotic and showed a much shortened growth as compared to plants of long-day exposure. This effect was more pronounced at 16° than at 28°. At both temperatures, symptoms of virus A and of viruses A and B together were less severe and those of virus B more severe under short day than under long-day exposures.

Since it had previously been determined that the incubation period of virus A was much longer at 16° than at 28° C., it was thought that the low concentration of the virus at 16° might be a result of the long period which the virus required to establish itself at 16° rather than a temperature effect after the virus was thoroughly established in the plant.

To test this possibility the following experiment was performed. Virus A and viruses A and B together were each inoculated to 30 young cabbage plants and incubated at a constant temperature of 22° for 12 days. They were then transferred to temperatures of 16° and 28°, half the number being placed at each temperature. Five days after removal to 16° and 28° an inoculation was made to tobacco to test the concentration of virus A. Twenty-five days later a second inoculation was made from the same plants to test the concentration after the lapse of 25 days. The results of the two inoculations are given in table 5. The concentration of virus A in plants at 28° was not significantly greater than the concentration in plants at 16° 5 days after removal from 22°, either in plants infected with virus A alone or in plants infected with both A and B viruses. However, after 30 days' incubation at 28° and 16°, the concentration of virus A in both A and A+B plants was much greater at 28° than at 16°. This would indicate that the long incubation period of virus A at 16° does not account for the low concentration of the virus at that temperature. Even after the virus is thoroughly established in plants, the concentration will rapidly diminish if plants are exposed to a lower temperature for any length of time.

TABLE 5.—*Concentration of cabbage virus A in cabbage plants infected with virus A alone and with viruses A and B together and incubated at 22° C. for 12 days before being placed at temperatures of 16° and 28°*

Half-leaf comparisons with virus—	Tempera-ture	5 days after being placed at temperatures of 16° and 28° [1]		30 days after being placed at temperatures of 16° and 28°	
		Comparison totals [2]	F value	Comparison totals	F value [3]
A	28°	994	2.45	897	53.89**
	16°	1,100		87	
A+B	28°	753	1.33	1,008	71.96**
	16°	675		72	
A	28°	970	.37	887	1.50
A+B		1,011		1,022	
A	16°	921	2.27	106	.079
A+B		819		75	

[1] In the first inoculation a randomized block design was used; each comparison was made on 2 plants of 3 leaves each. In the second inoculation a Latin square design was used; each comparison was made once on 12 different plants.
[2] Values given are local-lesion counts.
[3] **Highly significant.

One experiment was performed to determine whether viruses A and B would become established in plants at temperatures much lower than 16° C. Fifteen cabbage plants were inoculated with each of virus A and viruses A and B together and placed immediately in a coldframe where the temperature was held at about 5°. At the same time an equal number of plants was inoculated with each virus or viruses and placed at 28°. After 60 days' incubation the plants were removed from the coldframe to the 28° temperature, and an inocula-

tion was made from both groups of plants to test the concentration of virus A on the day of removal from the coldframe. During this 60-day interval the plants in the coldframe had made very little growth (two or three small leaves above inoculated leaves), and except for one inoculated leaf, they showed no evidence whatever of virus infection. However, after 48 hours at 28° systemic symptoms developed very rapidly. The A-infected plants developed symptoms typical of the virus A, and the A+B plants developed typical A+B symptoms. After a 30-day interval at 28° a second inoculation was made from both groups of plants in a manner similar to the first. The results of both inoculations are given in table 6.

TABLE 6.—*Concentration of cabbage virus A in cabbage plants infected with virus A alone and with viruses A and B together and incubated at 5° and 28° C. for 60 days and later at 28° for 30 days* [1]

Half-leaf comparisons with virus—	Temperature	After 60 days' incubation at temperatures 28° and 5°		30 days after removal of plants to 28° and 5°	
		Comparison totals [2]	F value [3]	Comparison totals	F value
A	28°	385	22. 07**	309	1. 38
	5°	3		263	
A+B	28°	609	56. 10**	359	. 11
	5°	0		372	
A	28°	424	. 62	250	. 48
A+B		488		277	
A	5°	3	. 001	191	. 71
A+B		1		224	

[1] In both inoculations a Latin square design was used; each comparison occurred once on 8 different plants.
[2] Values given are local-lesion counts.
[3] ** Highly significant.

It is noted that even after 60 days' incubation at 5° C. the concentration of virus A in systematically infected leaves was almost nil. However, when the same plants were exposed to a high temperature for 30 days, the virus concentration reached a level which was not significantly lower than that in plants which had grown the full 90 days at 28°. Apparently the virus was produced very slowly at this low temperature, but the rate increased rapidly when the plants were exposed to a higher temperature. Much of this condition was no doubt due to the reduce activity of the host. The very small amount of growth produced would indicate that the rate of host metabolism was very low.

In a previous paper (5) it was shown that the symptom severity of the black ring virus in relation to temperature was quite similar to that of virus A, while that of virus B was quite similar to that of the cauliflower mosaic virus. In the same report strain relationships in these two groups of viruses were established. Although both virus A and the black ring virus were favored by high temperatures, virus A was distinctly more severe than the black ring virus at high temperatures, whereas at low temperatures the reverse was true. The concentration of these two viruses at high and low temperatures was measured in plants infected with the viruses alone and in combinations with virus B and cauliflower mosaic virus, to ascertain if this measur-

able difference in symptom reaction was due to a reduced activity of the black ring virus at 28° and an increased activity at 16°. The cauliflower virus was included to see if it would react like virus B when in combination with virus A or the black ring virus.

The results of this experiment (table 7) show that the concentration of the black ring virus closely paralleled that of virus A, since both viruses occurred in significantly greater amounts at 28° than at 16°, when alone and when in combinations with virus B or the cauliflower mosaic virus. This indicates, as suggested in an earlier paper (5), that the effect of temperature upon any host-virus reaction depends upon the specific host-virus complex and may be due to the temperature effect upon the host, virus, or both. Although no half-leaf comparisons were made between virus A and the black ring virus, the results do not indicate that either occurs in significantly greater amounts than the other.

TABLE 7.—*Effect of temperature on the concentration of cabbage virus A and cabbage black ring virus in cabbage plants alone and in combination with virus B and cauliflower mosaic virus in the greenhouse*

Half-leaf comparisons with virus—	Temperature	Comparison totals [1]	F value [2]
A	28°	625	65. 91**
	16°	209	
A+B	28°	507	59. 13**
	16°	113	
A+cauliflower	28°	542	66. 55**
	16°	124	
Black ring	28°	498	51. 58**
	16°	130	
Black ring + B	28°	485	38. 76**
	16°	166	
Black ring+cauliflower	28°	514	48. 82**
	16°	156	

[1] Values given are lesion totals of 6 replicates in a randomized block.
[2] **Highly significant.

Virus B produces prominent and enduring symptoms at 16° C. and mild symptoms which are readily masked at 28°. To determine whether the concentration of this virus was affected by temperature, inoculum was taken from plants growing at each temperature and used to inoculate 100-plant lots of cabbage. An equal amount of comparable leaf tissue was used for each inoculation. When the virus extract was diluted 1:1, little difference was found in the number of plants infected. Since virus B has a very low tolerance to dilution, it was thought that by diluting the virus extract any difference in concentration at 28° and 16° could be more readily detected. Table 8 shows that on the basis of inoculations with extract diluted 1:50 the concentration was 100 percent greater at 16° than at 28°. Although this method of measuring virus concentration is probably less accurate than that used for virus A, it was the only method available since virus B is not known to produce local symptoms on any host. If virus B does occur in greater amount at low temperatures, as indicated in this one test, it would seem that the prominence of symptoms at low temperatures is related to the concentration of the virus. This condition has its counterpart in the reaction of virus A and black ring virus to high temperatures.

TABLE 8.—*Effect of air temperature on the concentration of cabbage virus B in cabbage plants*

Source of inoculum	Number of plants infected out of 100 inoculated	
	Extract diluted 1:1	Extract diluted 1:50
Cabbage at 28° C	94	45
Cabbage at 16° C	90	91

In all the preceding experiments virus A was found to occur in much greater concentration in cabbage plants growing at 28° C. than in plants growing at 16°. Since the rate of transpiration is probably greater at 28° than at 16°, a test was made to see whether the moisture content of the leaf tissue of plants at 16° was greater than that of plants at 28°, and whether in consequence the virus would be diluted upon maceration more at 16° than at 28°. Disks of tissue were cut from leaves at both temperatures and weighed immediately. These disks were taken at the same time and from opposite halves of the same leaves as the disks taken for the concentration tests. The results, given in table 9, show that there was little difference in the moisture content of leaves grown at the two temperatures.

TABLE 9.—*Moisture content of healthy and virus-infected cabbage leaves growing at 16° and 28° C.*

Plant infected with—	16° C.			28° C.		
	Fresh weight (gm.)	Dry weight (gm.)	Percent	Fresh weight (gm.)	Dry weight (gm.)	Percent
Virus A	[1] 1.115	0.117	89.50	1.085	0.112	89.70
Viruses A+B	1.113	.100	91.00	1.160	.100	91.30
Healthy	1.210	.146	87.90	1.070	.112	89.50
Total	3.438	.363	89.40	3.315	.324	90.20

[1] Weights given are for 10 tissue disks, 1 from each of 10 plants.

DISCUSSION

The concentration of virus A has been shown to decline as the symptoms of the disease recede with the gradual lowering of temperature in the late summer and autumn in the latitude of Wisconsin. Likewise limited evidence is presented that the concentration of virus B increased as the weather became cooler. The black ring virus showed the same temperature-concentration effect as virus A, to which it is closely related. Length of day had no measurable effect upon concentration of the viruses, but the symptoms of virus A alone and viruses A and B together were less severe, and those of virus B alone more severe, under short- than under long-day exposures. It is of particular interest to note that with virus A and with virus B there is a parallel increase of symptoms with increase in virus concentration.

Moreover, these two unrelated viruses appeared to increase or decrease in the same host in response to temperature without relation to each other. This, together with cross-immunity results presented in another paper (5), suggest the possibility that these unrelated viruses do not compete with each other in the process of conversion of normal proteins to the respective virus proteins after infection takes place. The cauliflower mosaic virus assumed the same role as virus B in combination with virus A. The black ring virus assumed the same role as virus A in combination with either the cauliflower mosaic virus or virus B.

There is nothing in the relation of virus concentration and temperature to explain the fact that at moderate temperatures (20° C.) the black ring virus causes greater necrosis on cabbage than virus A while at high temperature (28° C.) virus A causes greater chlorosis and mottle than the black ring virus.

It is not unlikely that many viruses vary in concentration with the temperature at which the host is grown. In fact, the temperature-concentration relation may be found to vary for the same virus in different hosts. The profound effect of temperature on virus concentration noted with the viruses studied in this investigation has led the writers to emphasize the importance of considering the temperature at which the host is grown when a given virus is being examined as to physical properties. This is particularly true since the original concentration of the virus extract may have a considerable influence on the point at which inactivation is noted as a result of dilution, aging, or exposure to given temperatures.

SUMMARY

The results obtained in this study are explanatory of observations on the epidemiology of the cabbage mosaic disease as it occurs in the Midwestern States. Symptoms of virus A appear in early summer when temperatures are high and remain severe until the cooler fall temperatures appear. The A symptoms then recede and symptoms of virus B attain prominence. Inoculations from field plants in late season yield virus A only in small amounts, and very often not at all, but they yield virus B in abundance. These conditions are no doubt due to a reduction in the rate of production of virus A and perhaps to an increase of virus B at low temperatures. Moreover, in the southern belt of States where cabbage is grown in winter and early spring virus B is more prevalent than virus A. In California Tompkins et al. (10) states that the black ring disease of cabbage occurs in the winter and is uncommon during the summer months. Although in these studies the effect of temperature on concentration of the black ring virus was found to be affected by temperature similar to that on virus A, it was earlier shown that the black ring virus produced more severe necrosis at low temperatures than did virus A. However, the failure of the black ring disease to occur in the summer months cannot be explained by the results obtained in these temperature studies.

Quantitative studies have been made to determine the effect of air temperature on the concentration of cabbage virus A, cabbage black ring virus, and cabbage virus B in cabbage plants. The effect of day

length on the concentration of cabbage virus A was also studied. Results obtained were subjected to statistical treatment.

Cabbage virus A and cabbage black ring virus were shown to occur in significantly greater concentration in cabbage plants growing at 28° C. than in cabbage plants growing at 16°, both when occurring in cabbage alone and together with either cabbage virus B or cauliflower mosaic virus.

The concentration of cabbage virus A in cabbage growing in a 15-hour day at either 28° or 16° C. was not significantly different from that in plants growing in an 8-hour day. This was true whether the virus occurred alone or together with virus B in the host.

When plants infected with virus A and viruses A and B together were incubated at 22° C. and later moved to houses of 16° and 28°, the concentration of virus A in plants at 16° fell significantly below the concentration in plants at 28°.

When plants were inoculated and kept at 5° C. for 60 days, the systemic build-up of virus A was practically nil. When the plants were moved from the 5° temperature to a temperature of 28°, the concentration of virus A after 30 days increased until it was not significantly different from that in plants which had grown the full 90 days at 28°.

No significant difference was found between the concentration of virus A in plants infected with virus A alone and the concentration in plants infected with both viruses A and B. It is suggested that the increased severity of symptoms of viruses A and B together over that of either virus alone results from the additive effect of each virus on the metabolism of the host.

Results obtained indicate that virus B occurs in greater amounts in plants grown at 16° C. than in plants grown at 28°, although the method used was less accurate than that employed in measuring the concentration of virus A.

The concentration of virus A in field plants progressively declines as plants grow into increasingly cool temperatures in the fall.

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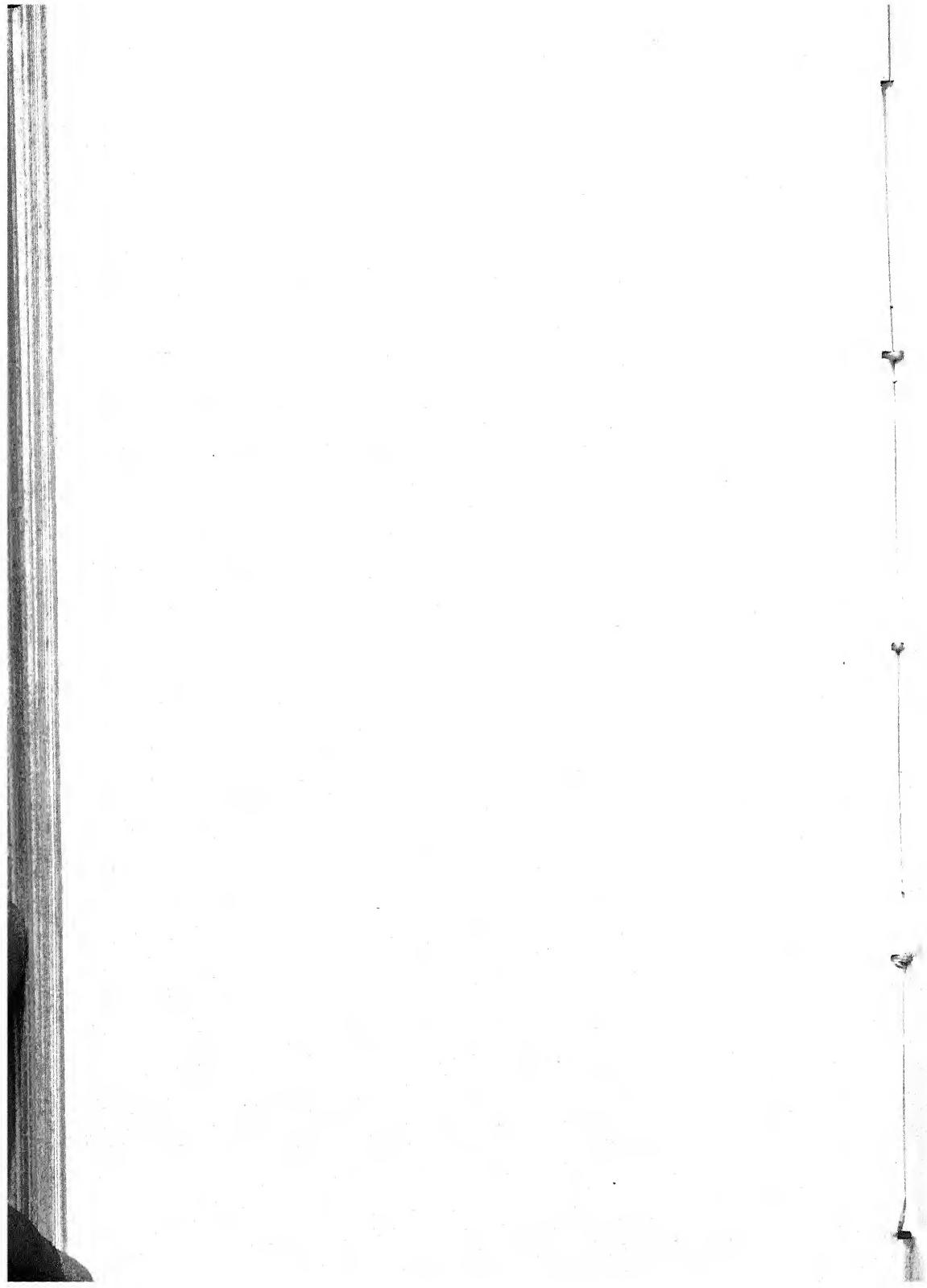
PHYSIOLOGICAL INTERNAL NECROSIS OF POTATO TUBERS IN WISCONSIN[1]

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INTRODUCTION

In the sandy, gravelly soils of Adams, Juneau, Portage, Waupaca, Waushara, and Wood Counties of central Wisconsin (fig. 1), and to some extent in Barron and Washburn Counties in the northwest, and in Washington County in the southeast, serious losses to the late
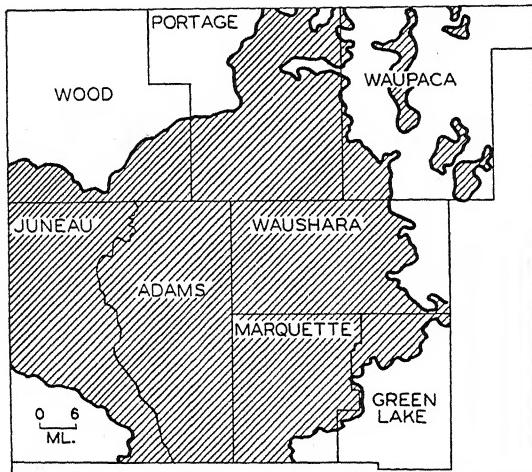

FIGURE. 1.—Sandy soil area of central Wisconsin shown by cross hatching.

commercial potato crop are sustained during certain seasons because of internal necrosis (physiological brown or rust spot) of the tubers.[3] This internal tuber disease condition greatly reduces the culinary, dehydration, and chip quality because the necrotic lesions remain as discolored, fibrouslike masses in the final product. The malady is most prevalent during seasons in which temperatures are above normal and precipitation below normal. Internal brown or rust spot was very prevalent in the State in 1937, 1939, and 1943, more prominent than usual in 1938 and 1942, and moderate in 1940 and 1941.

The present paper is a report of field and laboratory investigations which have been under way since 1938. Several preliminary reports of the work have been made (*1*, *2*).[4] Laboratory studies were con-

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ducted at Madison, Wis., and the field studies were carried out on Plainfield sand in the commercial potato area at Hancock, Waushara County, Wis. The occurrence of internal tuber necrosis in Wisconsin prior to the present investigation has been reported (*12, 13*).

METHODS AND MATERIALS

RECORDING INDEX VALUES

The method of calculating amount and degree of internal tube necrosis was similar to that used by the writers (*24*) in their analysis of scab resistance in potatoes. The tubers for examination (random sample of 100 or more tubers per replicate) were cut to expose as much of the internal necrotic tissues as possible, necessitating in some cases the cutting of tubers two or three times. Severity of necrosis was recorded in four arbitrary classes as follows: (1) Clean—no macroscopic necrosis; (2) slight—scattered flecks; (3) moderate—diffused irregular flecked areas; (4) severe—diffused and irregular large concentrated lesions. Tubers representative of classes 2, 3, and 4 are shown in figure 2. The index number 0 indicates all tubers free from internal necrosis, while 100 represents a case in which all tubers had severe internal necrosis. To reduce the error in recording class divisions, all classifications were recorded by the senior author. The Katahdin variety was used throughout all laboratory and field experiments, except varietal resistance tests, because of its susceptibility to internal tuber necrosis and its adaptability to growing conditions in central Wisconsin.

HISTOLOGICAL METHODS

Material for histological study was collected directly from a field planting of Katahdin potatoes at Hancock, Wis. All tissues selected were fixed immediately after they were cut by placing them directly in a solution consisting of 10 cc. of commercial 40-percent formalin, 5 cc. of glacial acetic acid, and 85 cc. of 50-percent ethyl alcohol. Tissues were evacuated under slight suction while in the fixative. The normal butyl alcohol schedule (*27*) was followed for dehydration. The tissues were embedded in tissue-mat paraffin with a melting point at 52°–54° C. Sections were cut 10 microns in thickness and mounted serially. The stain combination of safranine (1 percent in 95-percent ethyl alcohol) followed by fast green (saturated solution in absolute ethyl alcohol) was found to be most satisfactory for tissue differentiation.

SYMPTOMS

Internal necrosis is entirely a malady of the growing tubers; there are no manifestations of the disorder in aerial parts of the plant in the field during the growing season. Affected tubers show no external symptoms. The necrotic lesions appear translucent when thin tuber sections are exposed to transmitted light. Thicker tissue sections show the flesh to be marked wtih sparse amber-colored to reddish-brown specks converging into darker flecks or compact dark-brown irregular lesions scattered indiscriminately throughout the pith within the vascular cylinder (fig. 3, *A, B*). Less frequently they radiate from the pith toward the vascular ring (fig. 3, *C*) or appear to follow

the lines of the internal phloem.   In some cases affected tissues are confined to a necrotic mass (fig. 3, *D*), but as a rule they vary in size from mere specks to irregular blotchlike areas having a diameter of one-fourth to one-half inch.   Smaller lesions often coalesce to produce large irregular necrotic areas.

In cut tubers exposed to the air the internal necrotic tissues change little in color, although there is a slight increase in intensity of the rusty color already present.   The affected areas are firm, corky, and
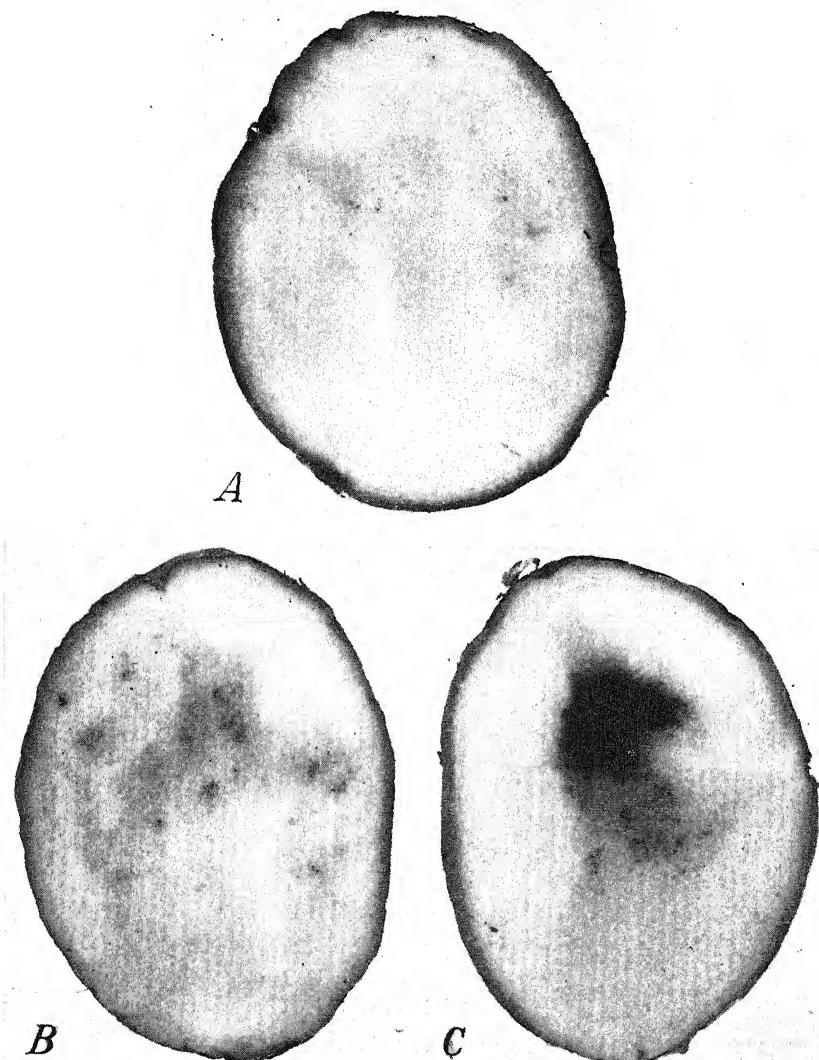

FIGURE 2—Index classes. Sectioned tubers showing representatives of three classes of internal necrosis used in determining the disease index.   *A*, slight; *B*, moderate; and *C*, severe.
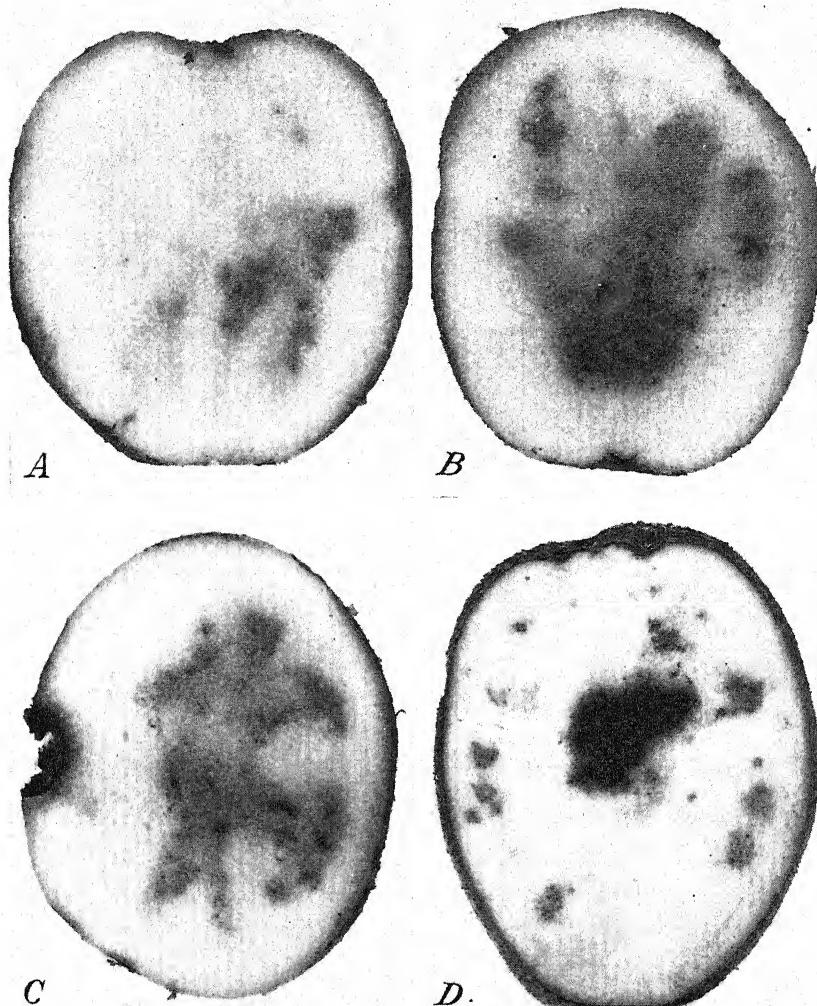
FIGURE 3.—Tubers of the Katahdin variety cut longitudinally, showing types of internal lesions.   A to D, Necrotic areas occurring indiscriminately throughout the pith tissues within the vascular cylinder.   Photographs taken at time of harvest.

leathery in texture.   Tissue break-down in the form of cavities or internal tuber decay following internal necrosis has not been observed in the field or in storage.

The internal necrosis under discussion is not reticulate and should not be confused with net or phloem necrosis of the potato tuber associated in certain potato varieties (Spaulding Rose, White Hebron, Irish Cobbler, and Green Mountain) with infection by the leaf roll virus (*10*); nor should it be confused with frost necrosis of the lesion type (*13*, *26*) or internal mahogany browning that occurs in the

Chippewa, Katahdin, and Cobbler varieties following long exposure to medium low temperature (*11*). Internal necrosis occurs, however, in the same areas in central Wisconsin as does the potato yellow dwarf disease. The tuber necrosis produced by the yellow dwarf virus is similar in many respects, both macroscopic and microscopic, to the nonparasitic internal necrosis considered in this paper (*14, 25*).

When tubers affected with internal necrosis are boiled or baked, the necrotic lesions remain as hard, dark-brown, corky masses in the softened pith tissues and the culinary quality is very greatly reduced. The type of internal discoloration referred to as internal necrosis is entirely distinct from the darkening or blackening of potatoes which sometimes occurs after boiling (*23*). The lack of distinguishable external tuber symptoms of this malady also increases greatly the cost in potato dehydration and chip manufacture because the internal disorganization is not ordinarily detected until the cutting stage.

The disease described above has much in common with types of nonparasitic internal necrosis of potato tubers reported from many parts of the world (*3, 5, 6, 8, 9, 16, 17, 19, 20, 21*); in fact, it may be identical with some of the cases noted. There has been no attempt in this study, however, to identify the Wisconsin malady with any described elsewhere. In certain of the previously described occurrences a correlation of the disease with high temperature and low moisture, as found in Wisconsin, has been recorded (*4, 5, 6, 15, 17, 19, 22*).

PATHOLOGICAL HISTOLOGY

Microscopical studies of affected areas of the tuber indicated distinct phases in the development and increase of the necrotic tissues in the internal and external medulla. The early stage of necrotic development is marked in apparently normal parenchyma by a slight darkening of the cell walls, which usually spreads along the walls from the corners of the cell. The abnormal discoloration was seen to affect two or more corners of the cell. The intercellular substance (middle lamella) in the region of the slightly thickened cell walls is also somewhat darkened (fig. 4, *A*). The second stage is characterized by the aggregation of granulate protoplasm against the cell walls and intensified thickening and discoloration of portions of the walls. This is accompanied by darkening of the walls of surrounding parenchymatous cells (fig. 4, *B*). In the third stage the affected cells tend to aggregate and collapse as a result of pressure exerted by abnormal division of contiguous cells. In addition, abnormal cell groups arise in the area of dividing cells, separated from similar affected groups, and become incorporated in the mass of dividing cells (fig. 5, *A*). In the final stage of tissue break-down there is a concentration of collapsed necrotic cells resulting from the increased pressure brought about by division of surrounding cells. The affected areas or cell groups are cut off or isolated from normal parenchyma cells by a cork meristem in contiguous parenchymatous tissue which subsequently forms a barrier between the normal and necrotic area and prevents further development of necroti c cells (fig. 5, *B*). There are no lysigenous cavities formed by disruption or disintegration of tissues in the internal necrotic pith areas or in the lateral branch medullary parenchyma. These observations of necrotic tissue development are somewhat similar to those reported for internal rust spot (*17*).
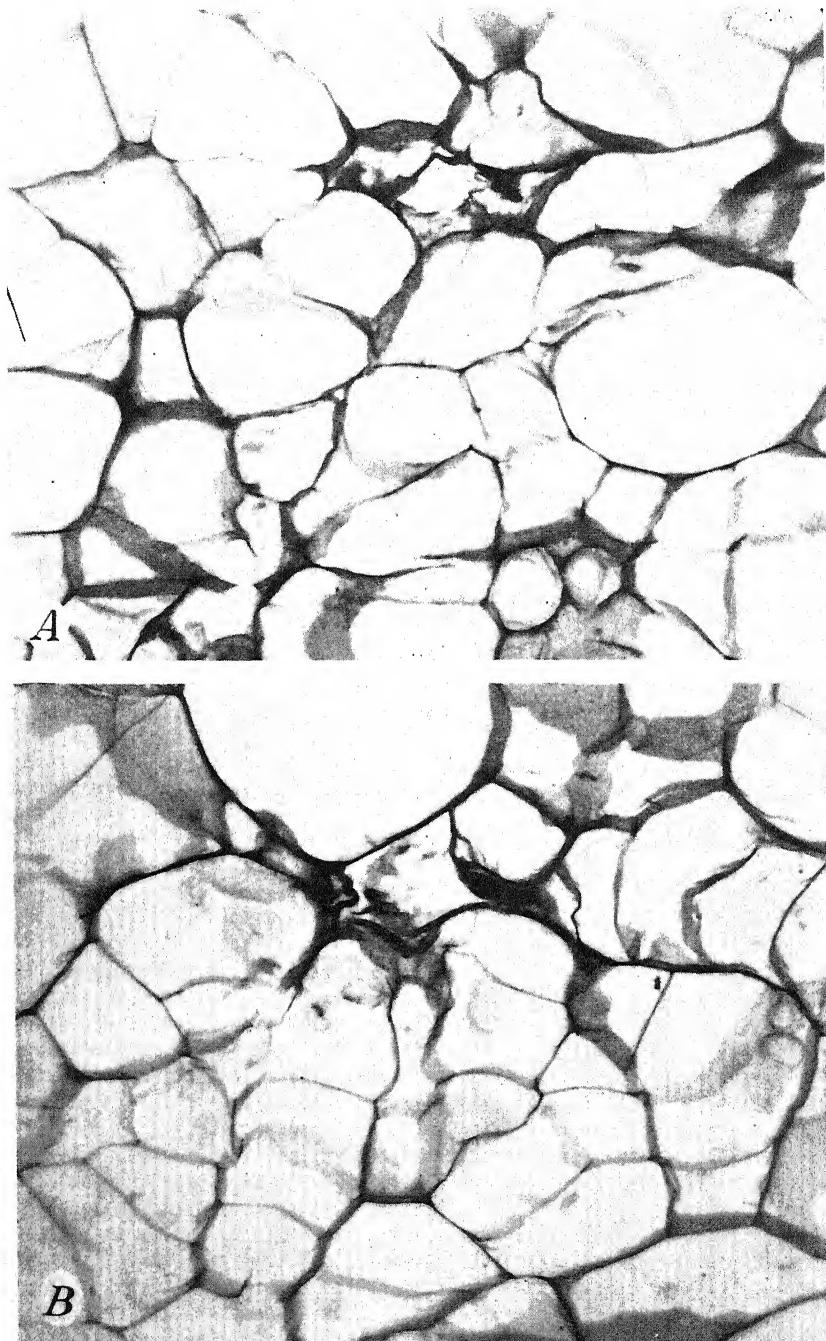
FIGURE 4.—Photomicrographs of tuber sections showing early stages in the formation of internal lesions: *A*, Early darkening of cell protoplast and thickening and darkening of cell walls; *B*, intensified cell-wall thickening and discoloration. X400.
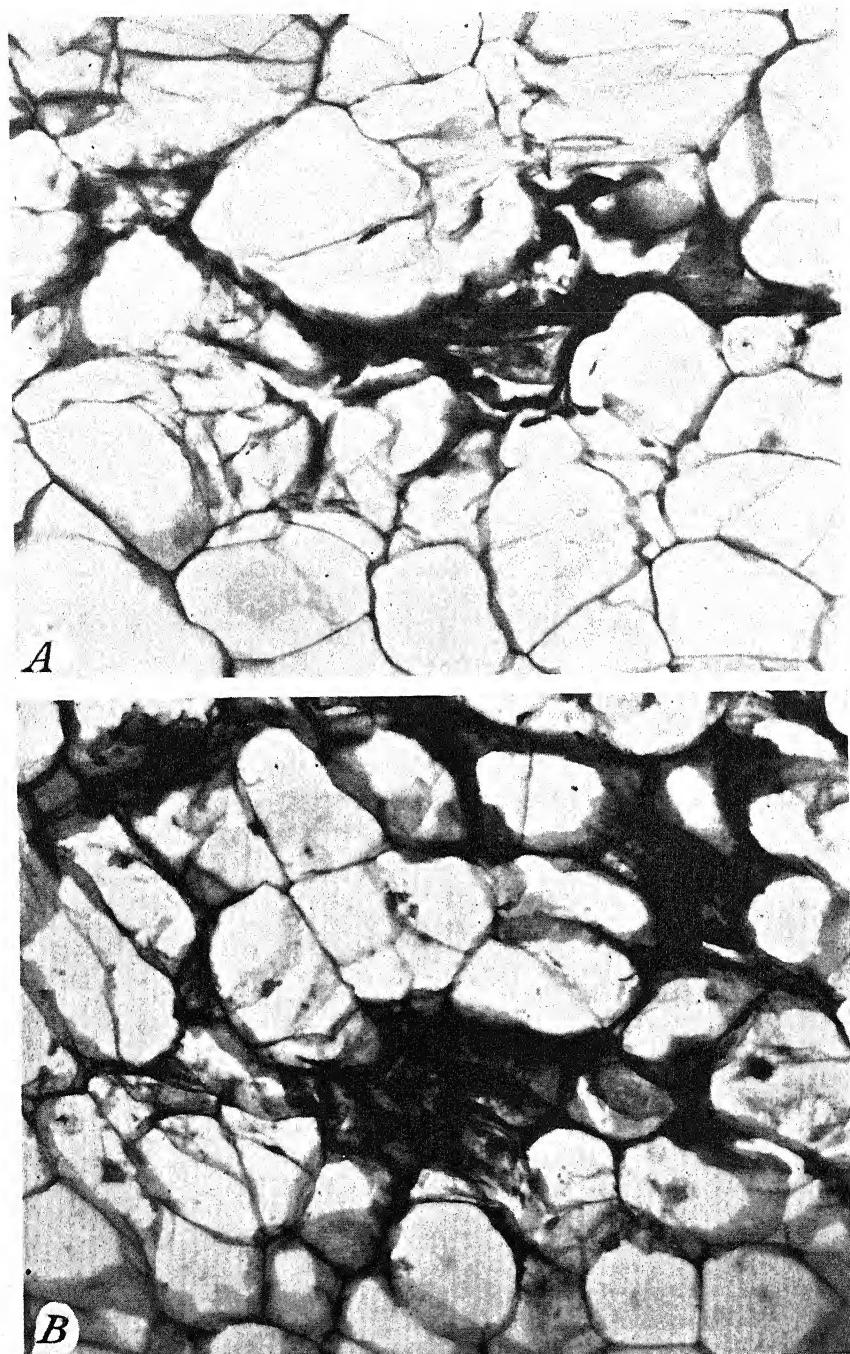
FIGURE 5.—Photomicrographs of tuber sections showing advanced stages in the formation of internal lesions: *A*, Aggregation of collapsed cells and increased division of surrounding parenchyma cells; *B*, increase of necrotic and collapsed cells to form lesions.   X400.

MICROCHEMICAL REACTION OF NECROTIC TISSUE

Mircochemical studies of affected and adjacent tissue were made on sections of freshly cut tubers, sectioned either by the freehand method or on the freezing microtome. Reactions were studied in both early- and late-harvested tubers. A considerable amount of suberin or suberized deposits was found in the cell walls of the freshly cut, discolored tissues. Walls of the disorganized cells of the lesions reacted to the suberin test in all cases where this type of tissue had been formed. The thickened walls of the disorganized peridermlike cells toward the periphery of the lesions also showed suberization. These outer cells became suberized, no doubt, as a reaction against the necrotic cell development in the early stages of disorganization and later, as the area increased, became incorporated in the corky meristem tissue. The greatest amount of suberized tissue was found in the cell walls contiguous to necrotic tissues. The presence of pentoses in the center portion of necrotic areas was shown, indicating that they accumulated in walls of the collapsed cells as they became thickened. No definite indication of formation of lignin was found.

Walls of cells in the compact necrotic areas and in the surrounding cells of the corky meristem did not give a positive test for cellulose. If cellulose does remain in the cell walls as they break down, its presence is apparently masked to a large extent by the accumulation of other substances. No positive reaction in tests for tanning or callose were secured in the dense necrotic areas; however, slight reactions indicated their presence in the outer cells of the lesions. A marked and consistent tendency for the thickened cell walls in and around the disintegrated area to show the presence of pectic substances was found.

Freshly cut necrotic areas showed the presence of proteins, dense cytoplasm, and anomalous crystals adjacent to the internal lesions. Where the lesions represented advanced stages and cells had collapsed and lost their identity, crystals were numerous. Such crystals were also found, but to lesser extent, in the outer cortical cells of affected tubers.

In cells composing the affected areas, starch grains were lacking, indicating a disturbance of metabolic processes. Starch grains occurred normally, however, in the adjoining parenchyma.

Necrotic lesions and contiguous parenchyma tissues gave a negative test for solanine, although tissues 1 to 2 cm. from the necrotic areas gave a positive test.

FIELD STUDIES

SEASONAL DEVELOPMENT OF INTERNAL NECROSIS

To secure adequate data as to seasonal development of internal necrotic lesions during tuber growth, the following field-plot experiments were carried out during the growing seasons of 1939 and 1940. A planting of 700 hills of the Katahdin variety was made on Plainfield sand at Hancock and received normal cultivation and frequent spraying with 5–5–50 bordeaux mixture and calcium arsenate. Tubers were harvested from 25 hills of each of 4 randomized replicates at weekly intervals during the season, beginning 73 days after planting. The necrotic index for each sample at each harvest date was determined as soon after collection as possible.

Comparative indices for the two seasons are given in table 1. There was a considerable increase in amount and severity of necrosis in the tubers as the season advanced. The difference in disease index between early- and late-harvest tubers for both seasons is quite marked. In general, the small-sized tubers of 1⅞-inch grade and under showed considerably less necrosis than tubers of U. S. No. 1 size. This is in agreement with observations of tuber size in relation to types of internal physiological tuber necrosis reported by others (*4, 15*). The correlation between tuber size and intensity and degree of necrosis indicates a relationship between growth processes and development of necrosis.

TABLE 1.—*Seasonal increase of internal tuber necrosis*

Number of days after planting	Disease index—		Mean index	Number of days after planting	Disease index—		Mean index
	1939	1940			1939	1940	
73	19	12	15.5	101	30	27	28.5
80	21	17	19.0	108	34	32	33.0
87	25	21	23.0	115	37	35	36.0
94	26	23	24.0	122	42	37	39.5

There was no measurable increase in the amount or severity of internal necrosis when tubers were cut and examined after a period of 8 months in storage at 40° F., following all harvests for the years 1939, 1940, 1941, and 1942. The data thus secured are further substantiated by data collected during the early and late part of the storage period in commercial warehouses and cellars in central Wisconsin during these years.

Moderately affected Katahdin tubers from the 1937 Plainfield sand plot planted at the Coddington station on muck soil for two successive seasons, 1938 and 1939, produced healthy, vigorous foliage and tubers free from internal necrosis and of excellent culinary quality. When the 1939 stock was again planted on Plainfield sand at Hancock in 1940 the tubers in the resulting crop showed an internal necrosis index of 37.5. Plantings of moderately affected seed at Madison in the field and greenhouse for five successive seasons, 1939 to 1943, inclusive, also yielded tubers free from internal necrosis. Similar examples of potato seed affected with certain types of internal physiological necrosis yielding normal tubers on being planted to muck or heavy soils have been reported (*3, 8, 16, 21*).

DEPTH OF TUBER IN THE SOIL IN RELATION TO INTERNAL NECROSIS

In a preliminary trial to ascertain differences in the occurrence and development of internal necrosis in relation to tuber depth in the soil, random samples of the Katahdin variety were taken from a commercial field planting shortly before harvest in 1937. Three groups of 100 tubers each were selected: (1) Tubers with not over 1 inch of soil cover; (2) tubers with 1 to 2 inches of soil cover; and (3) tubers with over 2 inches of soil cover. Sectioned tubers of the three groups showed great differences in both type and severity of internal necrosis. Tubers in groups 2 and 3 exhibited only diffuse internal necrosis of a type shown in figure 2, *B*, whereas group 1 showed a high percentage

of irregular concentrated lesions of a type shown in figure 2, *C*. The indices varied from 0.33 in tubers developing with over 2 inches of soil cover to 37.33 in those with a portion of the tuber exposed to 1 inch of soil cover.

In field trials conducted during 1938 to 1941, inclusive, the differences in severity and type of internal necrotic lesions between tubers developing with 1 inch or less of soil cover and those with more than 1 inch of cover were again quite consistent. Very few tubers showing irregular concentrated lesions were found in the two groups with soil cover over 1 inch, while, as recorded in table 2, there was a consider-

TABLE 2.—*Tuber depth in relation to internal necrosis*

Tuber sample	Disease index—					Mean index
	1937 [1]	1938 [2]	1939 [2]	1940 [2]	1941 [2]	
Group 1—exposed to 1 inch of soil cover	37.33	41.66	47.22	39.10	38.33	40.72
Group 2—1 inch to 2 inches of soil cover	13.33	16.66	18.30	13.28	14.29	15.17
Group 3—over 2 inches of soil cover	.33	.54	.71	.43	.47	.49

[1] 100-tuber samples at each depth.
[2] Mean of 4 replicates—100-tuber samples at each depth.

able decrease in the incidence of internal necrosis in tubers developing with over 2 inches of soil cover. In a series taken from a commercial planting in 1941, tubers from late-planted Chippewa and Russet Rural varieties showed the same relative differences in the incidence of internal necrosis in relation to the depth of soil cover during growth.

Internal tuber necrosis has not been observed in any variety under test planted as an early (late April or early May) crop on Plainfield sand. The late-maturing varieties, Chippewa, Katahdin, and Sebago now being planted early on a commercial scale in central Wisconsin, usually produce table stock of excellent quality with no internal tuber necrosis.

RELATION OF VARIETY TO INCIDENCE OF INTERNAL NECROSIS

A field study of varietal differences in incidence of internal necrosis was initiated in 1938 at Hancock on Plainfield sand. Trials were continued at this location during 1938 to 1943, inclusive. Early, midseason, and late-maturing standard varieties and recently introduced varieties were included. All plantings were made at the usual planting time (early June) for the late commercial crop in this area and received normal cultivation and spraying (5–5–50 bordeaux mixture and calcium arsenate) throughout each season. Indices of internal necrosis were determined each season shortly after the harvest of the crop in four replicates for each variety. The data and a tentative classification of varieties and strains are presented in table 3.

Significant measurable differences in the incidence of internal necrosis are to be noted when the 22 varieties under test are compared. Triumph, a standard early variety in the northern potato-producing areas of Wisconsin (Oneida and Vilas Counties) but not grown as an early variety in the central area, except under irrigation, has consistently shown no internal necrotic discoloration. Houma, a recently introduced variety, has shown a very low incidence in each

of the 3 years under test, exhibiting when affected only a slight diffused type of internal necrosis (fig. 2*B*). Rural New Yorker, Russet Rural, and Katahdin, on the other hand, have been among the most susceptible. The newer varietal introductions under test, Pontiac and Red Warba, were affected very much less by internal necrosis than Sebago, Earlaine, Mesaba, or Sequoia. It is important to note that Chippewa was distinctly less susceptible than Katahdin.

White-blossomed Cobbler, Russet Burbank, Spaulding Rose, and White Rose showed intermediate degrees of disease incidence. Green Mountain, Hundred Day Cobbler, and Columbia Russet are about as susceptible as Katahdin. Harmony Beauty was outstanding in its susceptibility to internal necrosis, exhibiting a severe irregular blotching necrosis (class 4, fig. 2, *C*).

TABLE 3.—*Reaction of potato varieties to internal necrosis*

Variety	Disease index—						Mean index	Number of years under test
	1938	1939	1940	1941	1942	1943		
Triumph	0	0	0	0	0	0	0.0	6
Houma	1	2	2				1.7	3
Pontiac			6	3	3	5	4.4	4
Red Warba		14	12	8	5	7	9.2	5
White-blossomed Cobbler [1]	11	10					10.5	2
Russet Burbank	18	10	14				14.0	3
Spaulding Rose		12	14	17			14.3	3
White Rose		18	10	15	19		15.5	4
Chippewa	20	14	14	27	22	19	19.3	6
Sebago			16	20	21	22	19.8	4
Irish Cobbler	23	22	18	31		19	22.6	5
Earlaine	27	22	16	28	24		23.4	5
Sequoia				30	32	29	30.3	3
Mesaba	36	30	26				30.6	3
Green Mountain	28	40	26	31			31.2	4
Hundred Day Cobbler	35	38					36.5	2
Columbia Russet	38	38					38.0	2
Rural New Yorker	38	32	31	56			39.2	4
Katahdin	41	46	34	30	39	47	39.5	6
Russet Rural	41	40	32	39	48	43	40.5	6
Pioneer Rural [2]	49	54					51.5	2
Harmony Beauty					52	57	54.5	2

[1] Natural seedling of Cobbler.
[2] Smooth mutant of Russet Rural.

It should be pointed out that the Sebago variety has distinct advantages as a late potato for the large commercial area of central Wisconsin, because of its intermediate resistance to internal necrosis, tolerance to late blight in both foliage and tuber, and high field resistance to the yellow dwarf virus (*14*).

Differences in varietal susceptibility to physiological internal necrosis have been reported for British, Dutch, and German varieties of potatoes (*4, 5, 7, 15, 18, 19*). In addition to 10 Dutch and 3 British potato varieties listed as resistant to a type of internal necrosis occurring in Holland, 3 American varieties, Burbank, Irish Cobbler, and Triumph were included (*19*).

SOIL MANAGEMENT AND THE INCIDENCE OF INTERNAL NECROSIS

Soil Mulching

In a study of the effect of soil mulch on the incidence of internal tuber necrosis, a series of field plots was laid out in randomized repli-

cated order in 1938 on Plainfield sand at Hancock, Wis. The following treatments were used: (1) Addition of 2 inches of rye straw; (2) 1 inch of muck soil cover; (3) shallow soil mulch provided by frequent cultivation; (4) no mulch; (5) soybean shade. The purpose of these soil-mulch treatments was to create, if possible, soil temperature differences around the growing tubers. In another treatment fresh-cut alfalfa was incorporated as green manure in the row before planting.

All mulch plots were planted and cultivated during the early stage of growth according to the current farm practices in the Hancock area. After the plants reached the early branching stage just prior to tuberization, the entire planting was weeded by hand before the application of the mulch treatments. The plots sown early to soybeans were weeded by hand during the entire season. Potato insects and foliage diseases were kept under control throughout the growing season by a general spray program.

The incidence of internal necrosis in the various treatments is shown in table 4. The most significant difference is to be noted when the straw mulch is compared with each of the other types. Incorporation of fresh-cut alfalfa as green manure and muck-soil mulch resulted in a slightly higher necrotic index than any of the other treatments.

TABLE 4.—*Internal necrosis index on soil management plots*

| Type of soil management | Disease index [1]— | | Mean index |
	1938	1939	
2-inch straw mulch	3.0	4.0	3.5
Soybean shade	38.0	--------	38.0
No mulch (weeded only)	48.0	36.5	42.2
Soil mulch (cultivation)	43.0	45.0	44.0
Muck-soil mulch	52.0	--------	52.0
Alfalfa as green manure	68.0	46.5	57.2
Straw as organic matter	--------	34.0	34.0

[1] Mean of 4 replicates.

Soil management studies were continued in 1939 with the exception of the soybean shade and muck-soil mulch. One set of organic matter plots was added, however, in the form of rye straw plowed under. It was thought possible that the low necrotic index recorded in 1938 where rye-straw mulch was applied could have been due to the action of some constituent in the straw in addition to the effect of reduced temperature and the maintenance of a higher, more uniform soil moisture. A significant reduction in internal tuber necrosis again occurred only in the case of the straw mulch (table 4). The fact that the rye straw as organic matter showed no benefit would indicate that the effect of straw mulch is only one of reducing soil temperature and retaining a higher level of soil moisture.

In 1938 soil temperatures were recorded twice weekly with a thermocouple at 3-inch and 6-inch soil depths during July, August, and September in all the plots. Although the biweekly records were somewhat inadequate, considerable variations were recorded in soil temperature as a result of differences in soil management. The greatest temperature differences recorded were those between the straw-mulch and the no-mulch plots. The mean soil temperature under the straw

mulch was lower by 12.1° F. at the 3-inch level and 10.8° at the 6-inch level than at corresponding depths in the no-mulch plots.

Continuous soil temperature records were also taken in 1938 at 1½-inch, 3-inch, and 6-inch soil depths in the no-mulch cultivated field plot. Recording thermometers were housed in shelter boxes about 15 inches above the soil surface with the thermometer bulbs buried in a horizontal position in the row at the desired depth, 6 to 8 feet away from the shelter so as to avoid any shading or radiation effect from it. A continuous air temperature record was also taken. The number of hours above 85° F. at 1½-inch, 3-inch, and 6-inch soil levels and in the air are given in table 5. It will be seen that there is a very wide difference in degree hours between the different soil depths, ranging from 227 hours at the 1½-inch depth to 110 hours at the 6-inch depth; 117 degree hours were recorded for the air temperature.

TABLE 5.—*Total weekly hours when temperatures were above 85° F. in the air and at 1½-inch, 3-inch, and 6-inch soil depths in a nonmulched, cultivated field plot, in 1938 on Plainfield sand at Hancock, Wis.*

| Week ending— | Hours of record | Hours above 85° F. | | | | Weekly precipitation |
		1½-inch depth	3-inch depth	6-inch depth	Air at soil level	
						Inches
July 24	168	50	18	10	11	0.44
July 31	168	31	19	12	13	.68
August 7	168	55	49	46	45	.75
August 14	168	37	23	20	21	.60
August 21	168	21	11	8	11	1.64
August 28	168	29	22	14	12	.38
September 4	(1)	4	8	0	4	.06
September 11	168	0	0	0	0	6.75
September 18	168	0	0	0	0	4.96
9-week total	1,512	227	150	110	117	15.26

[1] 120-week hours recorded.

Continuous temperature records obtained at 3-inch soil depth in nonmulched and straw-mulched field plots in 1939 and 1941 showed marked differences in soil temperatures. The 10-day air, soil, and rainfall records in table 6 were taken from field record sheets of July 23 to August 1, 1939, and July 24 to August 2, 1941. The mean soil temperature at 3-inch soil depth for the 10-day period in 1939 was 83.1° F. (nonmulched) and 74.2° (mulched) with a mean air temperature of 76.5°, and in 1941, 83.3° (nonmulched) and 70.0° (mulched) with a mean air temperature of 81.7°. The greatest difference in soil temperature, 26°, was recorded on July 24 and 31, 1941; however, 25° differences between nonmulched and mulched plots were not uncommon.

Although the application of the straw mulch was a means of reducing soil temperature, no direct evidence was secured which would indicate that the lower temperatures were more important in the reduction of internal necrosis than the conservation or uniformity of soil moisture. It is not possible to separate accurately the effect of soil temperature from that of soil moisture in field studies of this type.

TABLE 6.—*Air temperature record and soil temperature differences between non-mulched and straw-mulched potato field plots at 3-inch soil depth, Hancock, Wis., 1939 and 1941*

JULY 23 TO AUGUST 2, INCLUSIVE, 1939

| Date | Air temperature | | Soil temperature, 3-inch depth | | | | Precipitation |
| | | | Nonmulched | | Straw mulch | | |
	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	
	°F.	°F.	°F.	°F.	°F.	°F.	Inch
July 23	91	55	96	72	78	66	
24	94	64	94	74	69	70	
25	91	70	93	77	78	72	
26	98	65	99	77	81	72	
27	97	65	100	81	82	74	
28	87	62	82	74	75	70	
29	81	50	86	65	75	68	0.97
30	86	55	91	66	77	68	
31	97	64	95	72	79	70	
August 1	93	65	95	75	79	72	
Average or total	91.5	61.5	93.1	73.3	78.3	70.2	.97
Mean	76.5		83.1		74.2		

JULY 24 to AUGUST 2, INCLUSIVE, 1941

July 24	98	69	98	71	72	65	
25	98	66	94	70	72	65	
26	102	72	96	71	72	66	
27	97	73	91	75	72	67	
28	94	71	89	72	72	68	.58
29	100	76	96	72	74	70	
30	90	70	96	75	75	69	.10
31	88	69	101	71	75	69	
August 1	85	69	95	70	72	68	
2	91	56	98	66	73	65	
Average or total	94.3	69.1	95.4	71.3	72.9	67.2	.68
Mean	81.7		83.3		70.0		

No doubt a disturbance of water balance within the tubers in the form of diminished respiratory activity and altered cell growth plays an important role in the occurrence and severity of internal tuber necrosis. Tubers grown under straw mulch do not produce the knobby apical outgrowths or growth cracks common in tubers from the no-mulch plots and the culinary quality of straw-mulched tubers is very much improved, the tubers being more mealy in texture, of better flavor, and with much less tendency to discolor after boiling even after 5 months in storage.[5]

When mulched and unmulched tuber lots were held in a common storage for 5 months, tubers from the unmulched lot were much more shriveled and sprouted very much earlier than those from the mulched lot.

Soil Treatment

Various soil, fertilizer, and amendment treatments were conducted on Plainfield sand at Hancock in 1938 and 1939. These treatments included the application of hydrated lime and sulfur to modify the soil reaction; combinations of macroelements in the form of nitrogen,

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[5] The writers are indebted to the late Dr. W. E. Tottingham, Department of Biochemistry, University of Wisconsin, for making these tests.

phosphorus, and potash; microelements as soluble salts of boron, copper, iron, magnesium, manganese, and zinc incorporated in a complete fertilizer; and borax without fertilizer. In 1939 and 1940 soil treatments included various amounts of microelements alone in the form of salts of copper, manganese, and zinc. The initial soil applications were broadcast and disked in thoroughly on plowed ground about a month before planting. Plot planting, seasonal culture, and insect and foliage disease control were essentially the same as in the soil-management series.

The influence of various soil treatments on the incidence of tuber necrosis as expressed by the disease index is shown in table 7. The

TABLE 7.—*Soil treatment in relation to internal tuber necrosis*

Soil treatment	Pounds per acre	Disease index [1]—			Mean index
		1938	1939	1940	
Sulfur	1,000	35	38	-----------	36.5
0–62–0 fertilizer	200	39	37	-----------	38.0
Hydrate lime	2,000	49	36	-----------	42.5
8–8–15 fertilizer	800	58	29	-----------	43.5
Do.[2]	500	55	46	-----------	50.5
0–0–50	800	62	43	-----------	52.5
Borax	40	56	52	-----------	54.0
No treatment	-----------	48	43	-----------	46.0
Zinc sulfate	50	-----------	40	35	37.5
Do	100	-----------	42	32	37.0
Do	200	-----------	44	32	38.0
Manganese sulfate	100	-----------	45	34	39.5
Do	200	-----------	38	32	35.0
Do	300	-----------	38	31	34.5
Copper sulfate	100	-----------	50	38	44.0
Do	200	-----------	46	34	40.0
Do	300	-----------	41	31	36.0
No treatment	-----------	-----------	45	38	47.0

[1] Mean of 4 replicates.
[2] With additional microelements in the form of soluble salts of B, Cu, Fe, Mg, Mn, and Zn.

adjustment of soil reaction by the addition of hydrated lime and sulfur was readily secured. The initial soil reaction of about pH 5.5 was increased with the addition of 2,000 pounds per acre of hydrated lime to about 7.9 and decreased to pH 4.0 after the application of 1,000 pounds of sulfur. No significant difference in the incidence of tuber necrosis resulted at either pH. However, a definite variation in the severity of internal necrosis was noted when the cut tubers from the two ranges of soil pH were compared. Few tubers produced on the sulfur treated plots (pH 4.0) developed the severe internal necrosis of class 4 (fig. 2, *C*), whereas in the high alkaline soil (pH 7.9) a large percentage of this class was recorded. Partial control by various soil and amendment treatments have been reported by other workers but results have not been consistently successful (*15, 16, 19, 21*).

DISCUSSION

Internal tuber necrosis is an important factor in commercial potato production during certain seasons in the sandy soil areas of central Wisconsin and in many instances has been a limiting factor in the production of a marketable crop. Because of the fact that there is a lack of distinguishable external tuber symptoms of the malady it is

not ordinarily detected until tubers are cut in the final culinary stage, in the dicing stage of dehydration, or sliced in chip manufacture.

In the present investigation, while an attempt was made to correlate the occurrence of internal tuber necrosis with the more variable environmental soil factors, such as temperature and moisture, and the more stable factors, such as the soil type, organic matter, fertility, and soil reaction, only the gross differences in the soil mass were measurable. The conditions immediately surrounding the potato tubers and roots undoubtedly exert an influence on the occurrence of internal necrosis but are inadequately measured by present methods.

The incidence of internal tuber necrosis is significantly different at successive periods in the development of tubers, the necrotic index in late-harvested tubers being more than double that of the earliest. Tubers of the Katahdin variety harvested 73 days after planting showed a necrotic index of 15.5, exhibiting only the slight and moderate diffused type of necrosis, whereas after 122 days the necrotic index had increased to 39.5 with more irregular, large, and concentrated lesions present. This difference, together with the fact that the amount and degree of necrosis is more severe in larger tubers, indicates that the malady has its inception during the initial tuber development and increases during growth of the tubers. There is no increase in the incidence of internal necrosis during storage.

In relation to the tuber depth in the soil, approximately the same incidence of internal necrosis was observed at the respective depths studied over a period of five seasons. The mean necrotic index in tubers exposed to 1 inch of soil cover was 40.72, with 1 inch to 2 inches of soil cover 15.17, and in tubers with over 2 inches of soil cover 0.49. These differences may explain in part, at least, the high incidence of internal necrosis in certain varieties which have a tendency to set tubers near the soil surface. Green end tubers did not show a greater incidence or degree of internal necrosis than the tubers with 1 inch of soil cover.

Considerable variation in the relative susceptibility of the varieties tested was observed. The standard varieties most resistant to internal necrosis were Triumph, Houma, Pontiac, and Red Warba, with indexes of 0, 1.7, 4.4, and 9.2, respectively; Rural New Yorker, Katahdin, Russet Rural, and Harmony Beauty with indexes of 39.2, 39.5, 40.5, and 54.5, respectively, were the most susceptible. It is of interest to note that Pontiac and Red Warba, both having Triumph as one parent, are among the most resistant. Field trials are now under way to study possible inheritance in seedling crosses. The Chippewa and Sebago varieties, replacing the Russet Rural as late-maturing varieties in the central Wisconsin area, are about equal in their susceptibility to internal necrosis, being 19.3 and 19.8, respectively. The Sebago variety, however, is gaining in preference over the Chippewa as a late variety because, in addition to foliage and tuber tolerance to late blight it has a very high field resistance to the yellow dwarf virus. All varieties under test, planted as an early crop (late April or early May) at Hancock, were free from internal necrosis.

In soil-management studies, straw mulch had a definite, beneficial effect in reducing internal necrosis in all field tests. No direct evidence was secured, however, that would indicate that a reduction in soil temperature was more important than the conservation or uniformity

of soil moisture. It is quite possible that in the unmulched soil a lack of proper water balance causing respiratory disturbances within the tubers during their early formation, altering cell growth and development, play an important role in the occurrence and severity of internal necrosis. The evidence obtained in limited field tests appears to indicate that in lighter soils the fluctuating factors of soil temperature and moisture are of greater significance than differences in organic-matter content, soil reaction, or soil fertility.

SUMMARY

The symptoms of physiological internal necrosis occurring in potato tubers produced on the lighter soils under central Wisconsin conditions are discussed.

Anatomical symptoms of necrotic lesions usually are first noted as a slight darkening of the corners, walls, and protoplast of the cells in the parenchymatous tissues of the internal medulla. The intercellular substance is also somewhat darkened. This is followed by the accumulation of protoplasm against the cell walls with intensified thickening and discoloration of the walls. Abnormal division of adjacent cells tends to reduce the collapsed cells to specific necrotic areas.

Microchemical studies showed internal necrosis to be associated with suberization in all cases where this type of tissue was found. The presence of pentoses was indicated. No definite indication of the accumulation of lignin, cellulose, or solanine in the necrotic tissue was evident. Starch grains were lacking in cells composing the affected areas and in contiguous tissues.

The study of seasonal development and tuber depth in the soil showed that necrosis increased both in amount and severity as the season advanced and that tubers with 2 inches or more of soil cover were very much less affected than those more lightly covered. No increase of internal necrosis was observed in tubers during storage.

The standard potato varieties, Triumph, Pontiac, and Red Warba, are much more resistant to the malady than are Katahdin, Rural New Yorker, Russet Rural, or Harmony Beauty.

Soil management in the form of straw mulch consistently reduced internal tuber necrosis. The application of hydrated lime, sulfur, combinations of macroelements, microelements as soluble salts of boron, copper, iron, magnesium, manganese, and zinc alone or incorporated in a complete fertilizer, and borax without fertilizer, did not reduce the incidence of internal necrosis.

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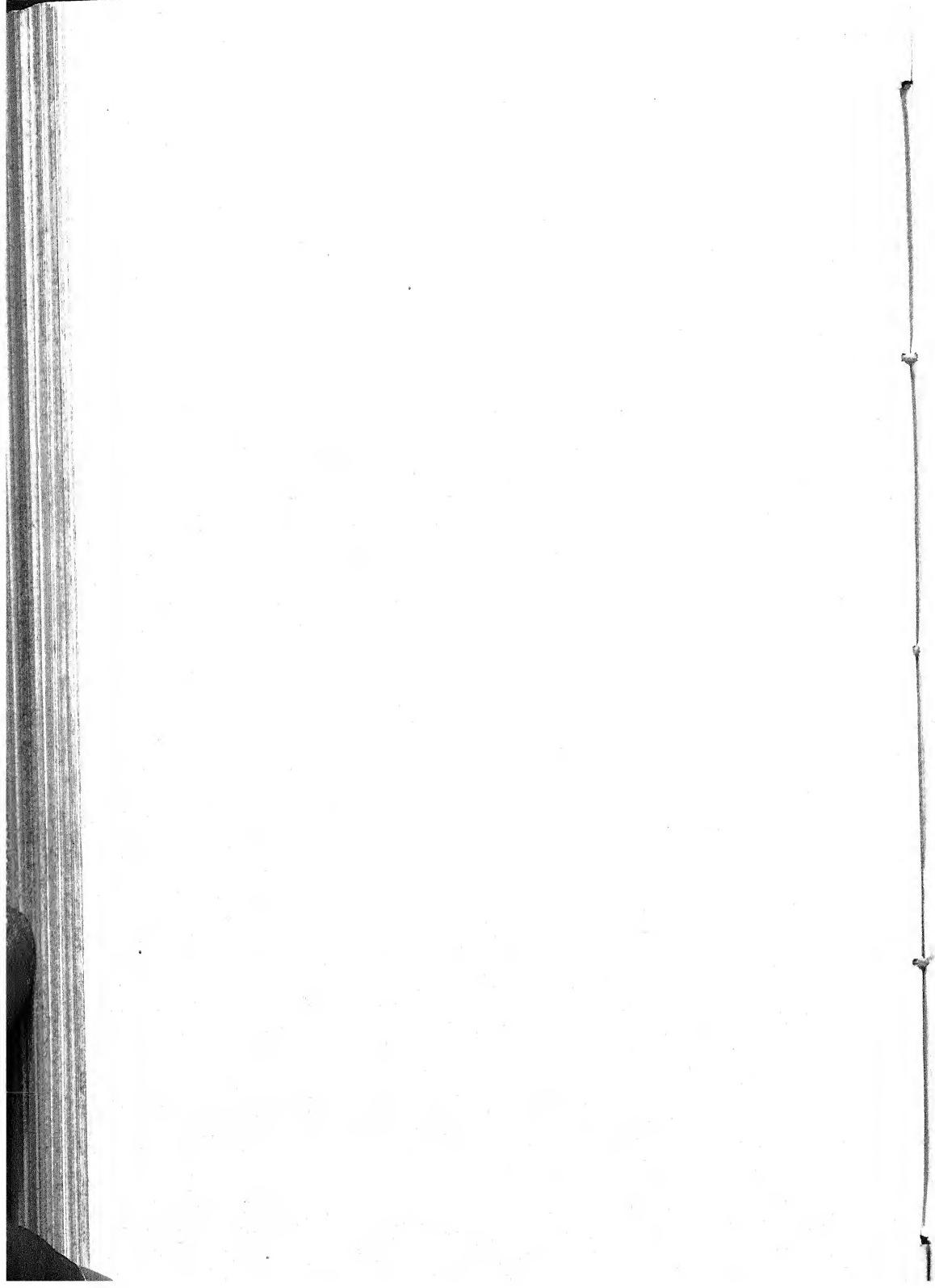
URINARY 17-KETOSTEROID EXCRETION BY BOARS [1]

By W. W. Green, *associate professor of animal husbandry*, and L. M. Winters, *professor of animal husbandry, Division of Animal and Poultry Husbandry, Minnesota Agricultural Experiment Station.*[2]

INTRODUCTION

Lines of inbred swine now being developed at the Minnesota Agricultural Experiment Station (*15*)[3] differ in the rate of sexual development and libido of the boars and the time of first estrus of the females. In the Minn. No. 1 line the boars began ranting early in the fifth month of age. The ranting was quite pronounced and apparently affected their rate of gain (*14*). While these boars seldom lacked in sexual desire, they did not exhibit marked secondary sexual characteristics. In other lines, especially the Market Lady and C_2, the boars looked and acted more or less like barrows until 200 to 250 days of age. Later they frequently lacked in sexual interest but, in contrast to the Minn. No. 1 boars, they developed strong secondary sexual characters such as heavy shoulder shields.

Urinary 17-ketosteroids were assayed from boars of various lines in order to find "normal" excretory values for various ages, possible differences among lines, and the association between 17-ketosteroids and sexual behavior.

REVIEW OF LITERATURE

Androgenic substances have been recovered from boar testes by Ogata and Hirano (*12*) and Hanes and Hooker (*3*). The last-named authors found that normal testes contain about twice the amount of androgens per gram of tissue as cryptorchid testes. Marsman (*9*) and DeReget, as quoted by Marsman, found, respectively, 90 and 134 mouse units of androgens per liter of boar urine. They also found 836 and 1,167 units of female hormone per liter of urine. Green et al. (*2*) assayed androgens of 48-hour urine samples of boars and reported a range of 0.21–2.09 capon units per sample. By the use of the Holtorff and Koch (*4*) technique they recovered 1,408–7,646 γ androsterone

equivalent of 17-ketosteroids from the 48-hour urine samples. They found a highly significant correlation of 0.88 between the results obtained by the capon comb and the Holtorff and Koch assay techniques. They also reported one 48-hour urine sample to contain 71.45 γ equivalent of estrone by the mouse vaginal smear technique.

METHODS

The methods for the collection of urine and the assay of 17-ketosteroids were the same as those described in an earlier report (*2*). The benzene used for extraction was thiophene-free and distilled before use. In order to standardize environmental conditions, boars representing the various lines were brought from the agricultural experiment substations to the laboratory immediately after weaning (8 weeks of age). They were kept in one pen so that all would have access to the same ration,[4] water supply, etc. For the urine collections, boars of similar ages were divided into groups of four, and the division was such that no two boars of the same line were in the same group. Collections were rotated so that each boar was sampled at 10- to 14-day intervals. The boars always remained in the collection crates exactly 48 hours. During that period they were fed a slop feed three times a day and watered twice daily. All results are given on the basis of 48-hour urine samples and are reported as γ equivalent of androsterone.[5] Statistical methods of Snedecor (*13*) were used for analysis of the data.

RESULTS

The benzene extracts possessed a red color similar to that of human urine extracts. Since some assays were completed before corrections were made for this residual color, studies were conducted to find whether the color had materially affected the results.[6] A nonsignificant correlation of -0.018 was found between the amount of residual color in an assay and the amount of 17-ketosteroid of that sample before correction. Correlation coefficients between the amount of urine per sample and the amount of 17-ketosteroids of samples (a) corrected or (b) uncorrected for color were not significantly different (185 samples). Since the amount of residual red color did not appear to affect the reliability of the results, all data reported hereafter are on the "uncorrected" basis.

EFFECT OF AGE AND SEASON OF THE YEAR ON 17-KETOSTEROID EXCRETION

The amount of 17-ketosteroids excreted varied with the age of the boars (fig. 1 and tables 1–3). Season of the year apparently affected excretion in two ways: (1) The season of the year in which the animals were farrowed, and (2) seasonal effects, as such, independent of the season of farrow. These latter seasonal effects appeared

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[4] Whenever buttermilk was fed as a portion of the protein supplement the urine foamed excessively when the HCl was added for hydrolysis, and this resulted in the loss of a number of samples. Only quantitatively recovered samples were used for assay.

[5] The authors are indebted to Dr. Erwin Schwenk, of the Shering Corporation, for supplying androsterone for colorimeter standardization.

[6] All extracts were brought to a 25-ml. volume after separation of the female hormone fraction and 0.2 ml. of the 25 ml. were used for each assay tube.

to be exerted even though the boars were of different ages at a specific calendar period.
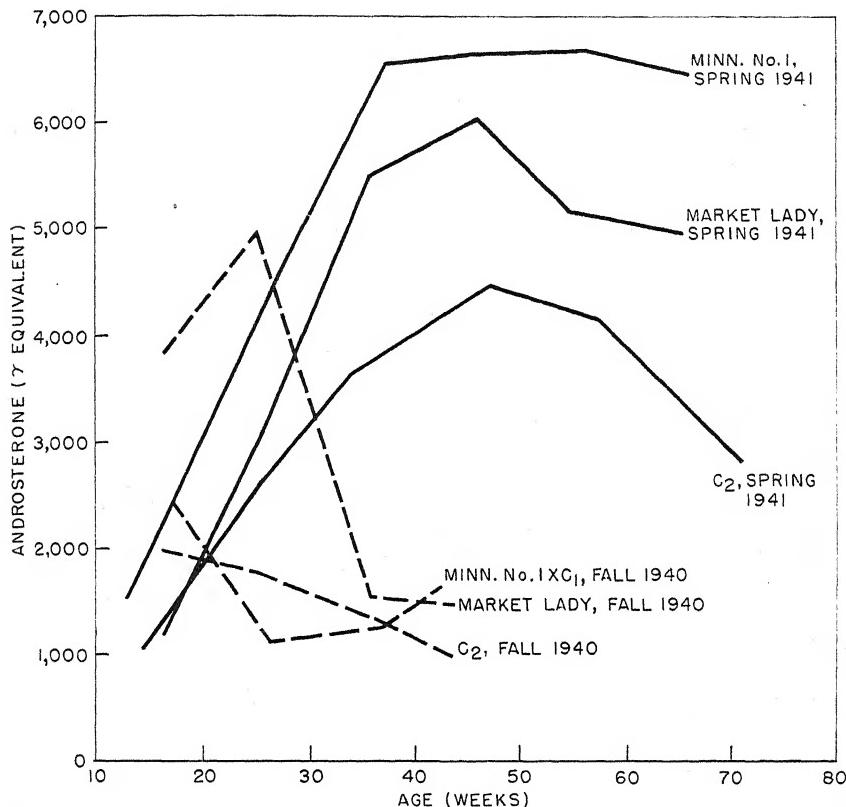

FIGURE 1.—Average 17-ketosteroid excretion during age periods indicated of boars of different ages, line of breeding, and season of farrow.

TABLE 1.—*Regression coefficients of 17-ketosteroid (y) on age (x) for the lines, ages, and time of farrow of fall- and spring-farrowed boars*

	Fall-farrowed boars			Spring-farrowed boars	
Age in weeks	Line	b_{yx}	Age in weeks	Line	b_{yx}
10–20	C$_2$	106	10–20	C$_2$	51
	Market Lady	388		Market Lady	88
	Minn. No. 1×C$_1$	208		Minn. No. 1	319**
21–30	C$_2$	−207**	21–30	C$_2$	309*
	Market Lady	−362		Market Lady	268**
	Minn. No. 1×C$_1$	−19		Minn. No. 1	182
31–40	C$_2$	157**	31–40	C$_2$	379**
	Market Lady	−27		Market Lady	−164
	Minn. No. 1×C$_1$	−40		Minn. No. 1	104
41–50	C$_2$	−9	41–50	C$_2$	−7
	Market Lady	−187		Market Lady	29
	Minn. No. 1×C$_1$	402*		Minn. No. 1	−525*

*Statistically significant at the 5-percent level.
**Statistically significant at the 1-percent level.

TABLE 2.—*17-Ketosteroid excretion of spring-farrowed boars by age and line of breeding*

Age in weeks	Line	Number of boars used	Number of samples	$\bar{s}$ [1]	$\bar{x}$ [2]	$\bar{y}$ [3]	Ratio $\bar{y}$ to $\bar{u}$ [4]
10–20	C₂	4	15	9.2	14.2	1,052	0.369
	Market Lady	4	14	24.8	16.0	1,199	.406
	Minn. No. 1	6	21	20.3	13.0	1,537	.349
	N. W. Black Star	1	1	21.0	13.0	1,084	.847
	All lines, total or average	15	51	18.3	14.8	1,293	.371
21–30	C₂	3	14	25.1	25.5	2,642	.576
	Market Lady	4	22	37.6	25.6	3,106	.906
	Minn. No. 1	3	12	39.7	27.0	4,550	1.036
	N. W. Black Star	1	2	40.5	29.5	5,976	1.594
	S. E	1	2	42.0	28.0	4,572	1.514
	C₁	2	2	30.0	26.0	2,130	1.910
	All lines, total or average	14	54	34.8	26.2	3,432	.781
31–40	C₂	2	8	36.9	34.0	3,636	1.136
	Market Lady	4	14	51.9	35.7	5,502	1.551
	Minn. No. 1	3	9	54.0	37.2	6,560	1.401
	N. W. Black Star	1	1	44.0	32.0	8,950	6.393
	S. E	1	2	56.5	38.0	4,872	1.686
	C₁	4	10	42.3	35.7	2,614	2.213
	All lines, total or average	15	44	47.4	35.8	4,772	1.539
41–50	C₂	2	5	55.6	47.2	4,483	1.657
	Market Lady	3	12	65.0	45.9	6,030	2.282
	Minn. No. 1	4	10	66.1	45.3	6,629	1.922
	All lines, total or average	9	27	63.7	45.9	5,965	2.020
51–60	C₂	1	2	69.0	57.0	4,189	1.614
	Market Lady	3	10	76.8	54.7	5,165	2.500
	Minn. No. 1	3	6	80.7	56.0	6,676	1.457
	All lines, total or average	7	18	77.2	55.4	5,560	1.876
61–76	C₂	2	5	88.6	71.0	2,845	.937
	Market Lady	3	5	90.2	64.8	4,945	1.003
	Minn. No. 1	3	4	94.3	65.5	6,450	1.755
	All lines, total or average	8	14	90.8	67.1	4,625	1.195

[1] $\bar{s}$ = Average season unit designation: The year was divided into 5 day unit intervals with the result that unit No. 74 coincided in calendar dates, May 1–5, with unit No. 1.

[2] $\bar{x}$ = Average age in weeks.

[3] $\bar{y}$ = Average γ androsterone equivalent per sample.

[4] $\bar{u}$ = Average milliliters of urine per sample.

Figure 1 indicates that during the age period of 10–20 weeks the fall-farrowed boars excreted more hormones than the spring boars. An analysis of variance made separately for the Market Lady and C₂ lines showed that the fall boars did excrete significantly more than the spring-farrowed ones.

Since the effects of season and age were necessarily intermingled, an attempt was made to separate their respective influences. Study of the dissimilarity in the hormone excretion of fall- and spring-farrowed boars was made on all lines. The significance of this difference was tested by comparing, in an analysis of variance, the shifts during 2-month periods in 17-ketosteroid excretion of spring and fall boars of approximately the same age. For example, the 1940 fall boars averaged 14 weeks of age when December assays were made; the average change in their hormone excretion from December to February was compared with that from July to September of 1941 spring boars which averaged 14 weeks of age at the time the July

TABLE 3.—*17-Ketosteroid excretion of fall-farrowed boars by age and line of breeding*

Age in weeks	Line	Number of boars used	Number of samples	$\bar{s}$ [1]	$\bar{x}$ [2]	$\bar{y}$ [3]	Ratio of $\bar{y}$ to $\bar{u}$ [4]
10-20	C$_2$	2	12	49.9	16.0	1,990	0.396
	Market Lady	3	15	48.7	16.2	3,833	.663
	Minn. No. 1×C$_1$	2	13	56.1	16.9	2,439	.454
	All lines (total or average)	7	40	51.5	16.4	2,827	.521
21-30	C$_2$	2	11	62.6	25.0	1,787	.306
	Market Lady	3	16	57.8	25.2	4,968	.861
	Minn. No. 1×C$_1$	2	7	68.7	26.4	1,115	.213
	All lines (total or average)	7	34	61.6	25.4	3,145	.553
31-40	C$_2$	2	9	77.6	36.1	1,318	.283
	Market Lady	3	16	76.0	35.6	1,524	.245
	Minn. No. 1×C$_1$	2	10	82.6	36.4	1,267	.408
	All lines (total or average)	7	35	78.3	36.0	1,398	.284
41-50	C$_2$	2	6	88.3	43.6	999	.300
	Market Lady	3	8	82.7	43.7	1,495	.254
	Minn. No. 1×C$_1$	2	4	91.7	42.5	1,612	.292
	All lines (total or average)	7	18	86.8	43.4	1,356	.274

[1] $\bar{s}$=Average season unit designation.
[2] $\bar{x}$=Average age in weeks.
[3] $\bar{y}$=Average γ androsterone equivalent per sample.
[4] $\bar{u}$=Average milliliters of urine per sample.

TABLE 4.—*Summary of comparisons of shifts in hormone excretion by spring- and fall-farrowed boars during comparable age periods*

Fall-farrowed boars			Spring-farrowed boars			F value
Time interval	Boars	Change in excretion	Time interval	Boars	Change in excretion	
	Number	γ		Number	γ	
Dec. 1940–Feb. 1941	5	2,947	July 1941–Sept. 1941	7	1,130	7.36*
Jan. 1941–Mar. 1941	6	−3,338	Aug. 1941–Oct. 1941	8	1,577	41.00**
Feb. 1941–Apr. 1941	6	−2,786	Sept. 1941–Nov. 1941	7	2,099	19.81**
Mar. 1941–May 1941	6	883	Oct. 1941–Dec. 1941	8	2,577	11.28**
Apr. 1941–June 1941	6	507	Nov. 1941–Jan. 1942	9	1,954	14.194**
May 1941–July 1941	6	−467	Dec. 1941–Feb. 1942	8	473	.797
June 1941–Aug. 1941	5	207	Jan. 1942–Mar. 1942	5	−289	.36

*Significant F value at the 5-percent level.
**Significant F value at the 1-percent level.

assays were made. Comparisons were made for seven different age periods, each period overlapping the preceding one by 1 month. The essential data, recorded in table 4, show that the change in hormone excretion with age was significantly different for these fall and spring pigs, which suggests a seasonal effect.

The differences in quantity of 17-ketosteroid just noted were not due to the amount of urine per sample. Urine excretion was analyzed in a manner similar to that used for the alternate month comparisons in the excretion of hormone. In only one of the seven instances was the difference in urine quantity significant (at the 5-percent level), namely, that for the April-June and November-January data. Quantity of urine decreased while 17-ketosteroid increased; so amounts of urine were not related directly to hormone values.

Data for studying seasonal effects on the rate of 17-ketosteroid excretion were secured by comparing fall boars which were sampled from December 1940 through August 1941 and spring boars which were sampled from June 1941 through August 1942. There were, therefore, two groups of boars approximately 6 months apart in age sampled during the same months of the summer of 1941 and similar months of separate years.

Tables 2 and 3 show a striking similarity of the effect of season on 17-ketosteroid excretion. After *s* values of 50–63 all lines showed a consistent decrease in excretion except the Minn. No. 1 $\times$ C_1 crossbred group which rose slightly after an average *s* value of 68. Although the Market Lady and C_2 lines differed during the period of 50–60 *s* units, each line was consistent in that the trends for the fall- and spring-farrowed boars of a line were similar. When the data for the C_2 and Market Lady lines for both fall and spring boars were combined for the age periods of 21–30 and 41–50 weeks, respectively, the regression coefficient of 17-ketosteroid and age with seasonal units (effects) held constant $(b_{yx \cdot s})$ was 87 while the regression coefficient of hormone on season with age effects held constant $(b_{ys \cdot x})$ was −145, which indicated that age and season were exerting different effects on hormone excretion during that season of the year.

Differences in the amount of urine per sample would not necessarily account for the seasonal influence on 17-ketosteroid excretion, because season of the year had little effect on the trend of the quantity of urine per sample. Quantity of urine was closely associated with age and followed similar trends for both fall and spring pigs.

RELATION OF QUANTITY OF URINE TO 17-KETOSTEROID PER SAMPLE

As age and season of the year apparently affect 17-ketosteroid excretion and as all of the correlations of hormone and urine quantities were positive (table 5), and in some cases more highly significant than age and season correlations, a study was made to find the association between urine excretion and hormones.

In order to test whether age or quantity of urine was most closely correlated with hormone excretion, the method of comparing correlation coefficients given by Baten (*1*) was used in all cases where the independent variables, age and urine quantity, were significantly correlated. *F* values were calculated from regression data in cases where the independent variables were not significantly correlated. In the case of the fall boars of ages 10–20 weeks, the correlations were significantly different, but the correlation of hormone and age was greater than that of hormone and urine. In no case did the urine quantity predict the amount of 17-ketosteroid better than age.

Correlation or regression coefficients of hormone quantity and amount of urine per sample may be misleading in indicating the trend of 17-ketosteroid values. In the case of the spring-farrowed boars in the age groups 41–50 and 51–60 weeks, the correlation coefficients of hormone and age, urine and age, and hormone and season were negative (table 5), while the coefficient of hormone and urine are positive. As both hormone and urine quantities are decreasing with age, they would be positively associated if the data involving hormones and urine were plotted on a correlation surface.

TABLE 5.—*Correlation coefficients of 17-ketosteroid excretion (y) and age (x), urine quantity (u), and season index (s) for boars of different ages*

Age in weeks	r_{yx}	r_{yu}	r_{ux}	r_{ys}
Spring-farrowed boars:				
10–20	0.445**	0.452**	0.374*	0.525**
21–30	.544**	.369*	−.016	.611**
31–40	.159	.308	−.114	.146
41–50	−.215	.775**	−.357	−.219
51–60	−.052	.672*	−.085	−.043
61–76	.825*	.779*	.755*	.761*
Fall-farrowed boars:				
10–20	.680**	.518**	.750**	.695**
21–30	−.341	.306	−.158	−.372
31–40	.088	.102	−.433*	.043
41–50	−.265	.212	.152	−.269

*Statistically significant at the 5-percent level.
**Statistically significant at the 1-percent level.

If the amount of 17-ketosteroids were dependent upon the amount of urine excretion, the ratio of hormone quantity to urine quantity should be constant. This ratio, however, increased from 0.37 to 2.02 for the spring-farrowed boars up to the age of 41–50 weeks (table 2) and decreased from 0.52 to 0.27 for the fall pigs of the same ages (table 3). The range in ratios was from 0.21 to 6.39 among the various lines and age groups. The lines of swine also varied in their hormone-urine ratios; the Market Lady spring boars had a higher ratio than the Minn. No. 1, which in turn had a higher ration than C_2 boars.

Further evidence of the lack of association between 17-ketosteroid excretion and urine quantity was shown by the fact that (1) trends in urine and hormone quantities were not similar when plotted against age; (2) that fall boars consistently excreted more urine on an average than spring boars but less hormone after 20 weeks of age; and (3) that the peak in the quantity of urine for both fall- and spring-farrowed boars occurred during the 21–30 week age period and 17-ketosteroid levels continued upward in the spring boars after that age.

These data seem to indicate that although the correlations between amount of 17-ketosteroid and amount of urine per sample were positive, and in some cases significantly so, these correlations are the result of circumstance and have little value in determining the actual trends of hormone excretion nor is the amount of 17-ketosteroid especially predicted by urine quantity.

LINE DIFFERENCES AND BOAR DIFFERENCES IN 17-KETOSTEROID EXCRETION

Boars of the Minn. No. 1 line farrowed in the spring consistently excreted more 17-ketosteroids than did spring-farrowed boars of the Market Lady or C_2 lines. In both fall and spring groups, the boars of the Market Lady line excreted more than the boars of the C_2 line (fig. 1 and tables 2 and 3). Data for the boars of other lines are given in tables 2 and 3 as a matter of interest, but the numbers are too small to permit definite conclusions.

Differences among the lines were not statistically significant at all ages. The degrees of freedom for an analysis of variance were keyed as follows: Differences between lines, among boars within lines, within boars within lines, and total.[7] Among the spring-farrowed boars, line differences, tested by boars within lines ($P = <0.01$), were found at

[7] *F* values for testing general line differences would result from testing the mean square of variance due to between lines by boars within lines. Line differences based only on the boars used in this experiment would result from testing between lines by within boars within lines.

21–30 weeks and line differences based only on the boars used were found at 31–40 weeks. Differences ($P = <0.05$) were indicated among boars within lines at 31–40 and 51–60 weeks. The fall pigs, 31–40 weeks, differed among the lines at the 5 percent level when lines were tested in the second manner. Variations among boars within lines ($P = <0.05$) were noted among the 10- to 20-week-old fall boars.

ANDROGEN AND 17-KETOSTEROID EXCRETION AFTER CASTRATION

A few stags were assayed by both the capon comb growth technique and the colorimetric method. Values for 17-ketosteroids were lower after castration although they were never absent. Results secured from one C_2 boar castrated at 325 days of age and assayed by the capon technique are shown in table 6.

DISCUSSION

The lines of swine used for this study are still undergoing development and are under constant change. As generations pass, variation in characteristics, such as degree of sex desire and earliness of sexual maturity, is continuous and problems prevailing during the years 1940 and 1941, although still present, appear in different intensities, in different lines, or in both. The results obtained in this study are based upon the conditions prevailing during the years the lines were sampled. Whether or not a second sampling of the lines would give the same results is, of course, open to question; however, that does not necessarily detract from the importance of the implications of this study.

The trend in the excretion of 17-ketosteroids by the spring-farrowed boars of different ages was similar in pattern to the excretion of androgens in human urine reported by Nathanson et al. (*10*), Neustadt and Myerson (*11*), and others. It was also similar to the change in the amount of androgens in bull testes of different ages (Hooker, *5*). The quantities of hormones excreted rose steadily from sometime before puberty until sometime afterwards. The decrease in excretion after a peak had been reached is similar to what occurs in bulls and in human beings. Since season was apparently exerting some effect about the time of the decline in hormone excretion, no definite conclusion could be reached as to whether the decline resulted from the influence of age or of season.

TABLE 6.—*Excretion of androgenic substances (measured in capon units) by a C_2 line boar before and after castration*

Dates of sample collection before castration	Androgenic substances	Date of castration	Dates of sample collection after castration	Androgenic substances
	Capon units			*Capon units*
August 21–23, 1940	1.64	November 23, 1940	January 13–15, 1941	0.39
September 25–27, 1940	1.08		January 20–22, 1941	0
			January 26–28, 1941	0

Two possible effects of season were noted; the spring-farrowed group excreted more 17-ketosteroids after 20 weeks of age than the fall-farrowed group. The excretion curves for the two groups were dissimilar, but those for all boars of one season's farrow tended to follow the same pattern. This indicated that some factor was exerting a similar influence on all the boars. Whether this was some phenomenon associated with the season of the year, or whether it was due to nutritional causes was not determined. All the boars were fed as nearly the same rations and treated as nearly alike as possible. In both spring and fall boars there was a reduced excretion of hormones in the latter part of February and early March. This was true even though the boars were 6 months apart in age and were sampled in different years. As the ration and management were constant throughout the year, it would seem reasonable to assign some of the effects observed to seasonal causes.

In view of this possible effect of season on 17-ketosteroid excretion, the data of Womack and Koch (*16*) are of interest. They found indications of possible seasonal differences in the androgen content of ram testes. It was slightly higher in August, October, November, and March than in January, February, and April. Rams will breed at any season of the year, but usually ewes will breed only in the fall. It may well be that seasonal changes in androgen production occur in the male of some farm animals even though it is not expressed in changes in the degree of sex drive.

The differences in the amount of 17-ketosteroid excreted by the various lines were not significant at all ages, but the consistency of the differences and the relative position of the lines is of considerable interest. With these animals, as with other species reported in the literature, the willingness of a particular individual to copulate was not necessarily correlated with sex hormone excretion. However, when the excretion of 17-ketosteroids was compared with the sex behavior of boars *by lines*, there seemed to be a rather close association between the two functions. As previously mentioned, the boars of the Minn. No. 1 line began to rant earlier and to a greater degree than the Market Lady or the C_2 boars. They also possessed more libido throughout life. In like manner the Market Lady boars preceded the C_2 boars in time and degree of sexual maturity as shown by sex drive. Differences among the lines in the age at which ranting or sexual desire appeared coincided quite well with the ages at which the regressions of hormone on age became significant as shown for the spring boars in table 1.

Differences among the lines in sex drive and response of somatic tissue is interesting in view of the work of Hooker (*6*), Hummel (*7*), and Kosin and Munro (*8*), who have shown that some tissues may be more responsive to androgenic stimulation at the time of puberty than before or after it, and that strains of mice and chicks showed a different response to pregnancy urine and androgens, respectively. The expressions of sex drive and of secondary sexual (somatic) characteristics in the Minn. No. 1 boars were the reverse of those in the Market Lady boars. Perhaps the ability of the somatic tissues and nervous system to respond to 17-ketosteroids at a given age varies among the lines in such a way that the response is not maximal at

the time of puberty in all cases. This might result in a lower response at a later age because of possible higher threshold-response values in older animals. On the other hand, the genetic constitution of the lines may affect the internal environment of the boars in such a way as to limit the response to 17-ketosteroids as well as determine the reactions at the time of puberty.

SUMMARY

Urinary 17-ketosteroid excretion by boars of different lines of inbred swine has been assayed from 48-hour urine samples. The residual red color of the benzene extracts was not found to have a material effect on the results of the assays. Correlations between the amount of urine per sample and 17-ketosteroids per sample were always positive and in some cases significant; however, the quantity of urine apparently had no real effect on the 17-ketosteroid values.

A sharp rise in 17-ketosteroid excretion at the time of puberty was not found. One stag excreted sufficient androgens to be assayed by the capon comb technique 58 days after castration but not 68 days after the operation.

Some effects of the season of the year on hormone excretion were noted.

The amount of 17-ketosteroids found in boars' urine varied with age and line of breeding. When compared on the basis of sexual behavior of the boars by lines, an association was found between the amount of hormone excreted and the sexual behavior of the boars. Some of the line differences in sex drive and somatic responses to the hormones were discussed.

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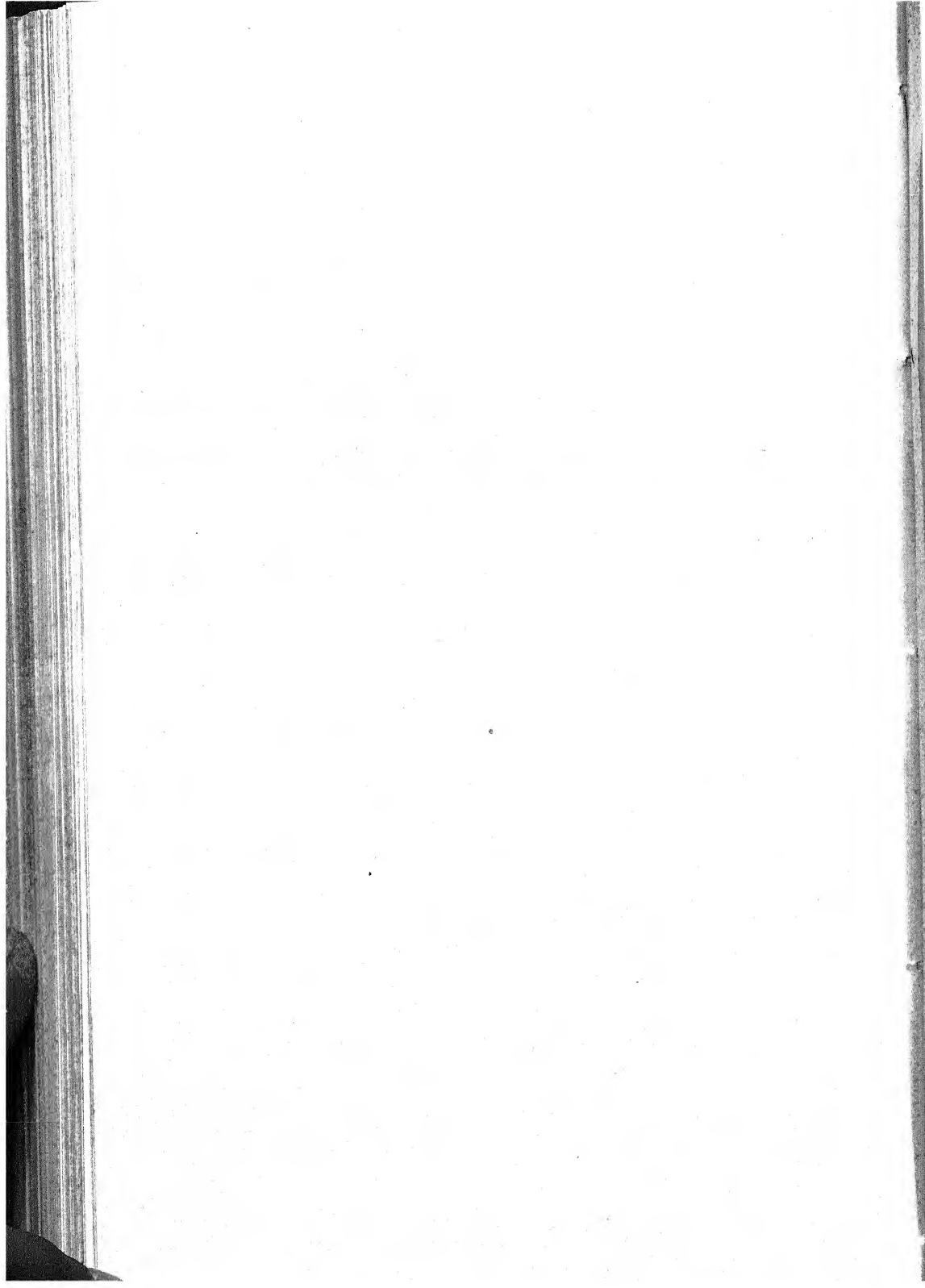
INFLUENCE OF SOIL MOISTURE CONDITIONS ON APPARENT PHOTOSYNTHESIS AND TRANSPIRATION OF PECAN LEAVES [1]

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INTRODUCTION

An optimum supply of soil moisture is an important factor in the production of pecans (*Carya illinoensis* K. Koch; syn., *C. pecan*). Nevertheless, it is not uncommon for the moisture in orchard soils to vary from inadequate to excessive amounts during the same season. For example, in the early spring of 1942 the soil moisture in certain parts of the orchard of the United States Department of Agriculture Pecan Field Station at Brownwood, Tex., had decreased to the wilting point owing to lack of rain, whereas in late spring the soil in some parts of the orchard was saturated for as long as 5 weeks owing to excessive rainfall and inadequate drainage.

Such extremes in soil moisture may be expected to interfere materially with the normal functioning of vital processes of plants, thereby influencing their vigor and productivity. Heinicke and Childers (7)[2] and Schneider and Childers (11) demonstrated the deleterious effects of low soil moisture on photosynthesis and transpiration of apple leaves. Childers and White (3), Boynton (2), Childs (4), DeVilliers (5), and others showed harmful effects of low oxygen content in the soil, as a result either of poor aeration or of flooding, on the growth and metabolism of apple trees. The experiments herein reported were designed to determine the effects of various soil-moisture conditions on the fundamental processes of photosynthesis and transpiration of pecan foliage and to ascertain the degree of injury, if any, caused by unfavorable quantities of soil moisture and the rate of recovery of the foliage from such injury.

MATERIALS AND METHODS

MATERIALS AND EXPERIMENTAL PROCEDURE

The procedure described by Heinicke and Hoffman (8) was used to determine photosynthesis. The leaf cups, or assimilation chambers, were similar to those designed by Heinicke (6) as adapted for use with pecan leaves by Loustalot and Hamilton (10). Transpiration was measured by passing the air through a dehydrating agent before

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it reached the carbon dioxide absorption towers and determining the increase in weight due to the water absorbed thereafter.

Two experiments were conducted during the season of 1942: experiment 1, with seedlings growing in coarse sand; and experiment 2, with seedlings growing in heavy soil. All plants were started in early spring from selected seed of the Burkett variety. On April 15 lots of six seedlings each were selected for uniformity of size and vigor and were transplanted to 5-gallon glazed crocks filled with coarse sand, one lot to each crock. The sand was prepared for use by washing it with a 1-percent solution of hydrochloric acid and thoroughly flushing it with tap water. Drainage was provided by a 1-inch hole near the bottom of the crock. Hoagland and Arnon's (9) nutrient solution diluted to one-fourth strength was supplied to the plants at regular intervals or as needed. The seedlings used in experiment 1 continued to grow in the coarse sand, which had a wilting coefficient of 1 percent and a field capacity of approximately 6 percent. For experiment 2 a brown silty loam of the Catalpa series, having a wilting coefficient of 12.2 percent and a field capacity of approximately 34 percent, was used instead of the sand. On July 28 the seedlings were transplanted from the crocks of sand to crocks of this soil, where they were allowed to grow for 29 days before the experiment was started.

General procedures for the two experiments were similar. In each, three crocks of representative plants were placed on a bench outside the laboratory. The plants in one crock were designated as the check plants, those in a second crock as the flood plants, and those in a third crock as the drought plants. In each experiment, one mature leaf on each of two plants in each crock was selected for similarity in position and exposure. Usually two determinations of photosynthesis and transpiration were made daily, one in the morning and one in the afternoon. The morning determinations began at 8 a. m. and ended at 11 a. m.; the afternoon determinations began at 12 m. and ended at 3 p. m.

For periods ranging from 4 to 13 days before the treatments, the rates of photosynthesis and transpiration were determined, the soil moisture in all pots being maintained at approximately field capacity so as to establish the ratio, or normal relation, of the rates of the test plants to those of the check plants.

In the crocks containing the drought plants the sand or soil was allowed to dry out during a given period, whereas in those containing the check plants the moisture was maintained at or near field capacity. Determinations of carbon dioxide and of transpiration were continued during the drought periods until a critical point was reached; then the sand or soil was watered to field capacity, and determinations of photosynthesis and transpiration were continued for several days in order to observe the rate of recovery of the two processes from the effects of the drought. Samples of sand or soil for moisture analysis were taken at frequent intervals (daily during the critical periods), in order to determine the rate of drying of the soils.

After the calibration, or pretreatment, period the drainage hole in the crocks containing the plants to be flooded was plugged and dilute nutrient solution was added until the level of the solution was 1 to 1½ inches above the surface of the sand or soil. The moisture that evaporated was replaced alternately with tap water and with dilute

nutrient solution, so that the level of the solution above that of the sand or soil was maintained. Determinations of photosynthesis and transpiration were continued on leaves of the flooded plants until definite trends in the rates, due to flooding, were evident or until a critical point was reached, after which the excess moisture was drained off. After the draining, further determinations were made in order to observe the rate of recovery, if any, from the effects of flooding.

At the time the flooded plants in experiment 1 were drained (August 23), one-half of the seedlings in each of the three treatments, check, drought, and flooded, were harvested for ash and total organic nitrogen analysis. The remaining plants were harvested on September 18. At each date of harvest entire plants were removed from the pots, the leaves were detached from the stems, and all tissues were placed in a drying oven at 105° C. for 24 hours; after this they were ground in a food mill and stored in aluminum cans until analyses were made. No analyses of plants were made in experiment 2. The official methods of analysis (*1*) for ash and for total organic nitrogen were used.

The percentages of soil moisture were determined from the loss in weight of soil dried for 24 hours at 105° C. The wilting coefficients were determined as the percentage of moisture remaining in the soils at the time of permanent wilting of sunflower plants growing in the soils. The soil samples were obtained by driving a metal tube one-half inch in diameter to the bottom of the pot, in the area occupied by the four seedlings not included in the test. Since six seedlings with well-developed root systems (see fig. 3, *A–C*) were growing in each pot, the soil was thoroughly permeated with roots. The sample of soil taken probably was as representative of the entire soil mass as could be obtained under the conditions without undue injury to the roots of the plants being studied.

METHOD OF CALCULATING RESULTS

Apparent photosynthesis was calculated as the average amount, in milligrams, of carbon dioxide assimilated per hour by 100 cm.^{2} of dorsal leaf surface; and transpiration as the average amount, in grams, of water lost per hour by 100 cm.^{2} of dorsal leaf surface.

The normal, or expected, rate of apparent photosynthesis was calculated as follows: The average number of milligrams of carbon dioxide absorbed per hour by 100 cm.^{2} of the proposed test leaves was divided by the average number of milligrams absorbed per hour by 100 cm.^{2} of the check leaves. The quotient was multiplied by 100 to obtain the percentage relation between the rate in the proposed test leaves and that in the check leaves. This percentage relation was considered as the rate which the proposed test leaves would be expected to maintain if they were not affected by the treatment, or 100 percent of the rate. Thereafter the percentage deviation from the expected rate in test leaves was calculated by multiplying the number of milligrams of carbon dioxide absorbed by the check leaves by the average percentage relation established between the check and test leaves before the treatment was begun. From this value, which represents the theoretical normal rate of the test leaves, the actual number of milligrams of carbon dioxide assimilated by the test leaves was subtracted, and the difference was then divided by the expected rate and multi-

plied by 100 to obtain the percentage deviation from the expected, or normal, rate.

Calculations of transpiration rates were similar to those for photosynthesis.

RESULTS

SEEDLINGS GROWING IN SAND

EFFECTS OF DROUGHT

The data in figure 1 show that the rates of photosynthesis of pecan seedlings growing in sand fluctuated somewhat around the average
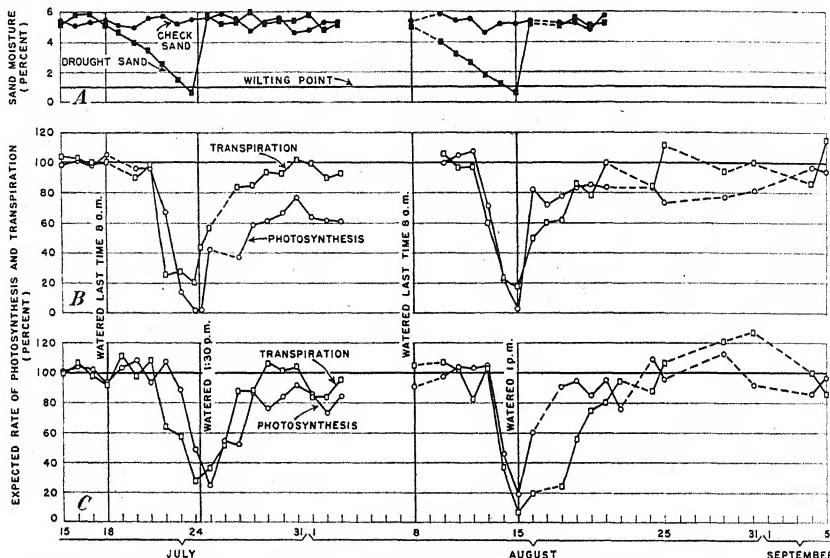

FIGURE 1.—Effect of drought on photosynthesis and transpiration of leaves of pecan seedlings growing in sand: *A*, Moisture percentages in sand; *B* and *C*, photosynthesis and transpiration (afternoon and morning determinations, respectively) expressed as percentage of expected rate.

rate during the 4-day calibration period (July 15 to 18). By the morning of July 22, the soil moisture was only 1.5 percent above the wilting point and the rate of transpiration was two-thirds of normal but photosynthesis was not appreciably affected. During the afternoon the rate of transpiration dropped sharply and photosynthesis was only two-thirds of normal. In general, the rates continued to drop until July 24, when the plants were watered again.

The young leaves of the test plants wilted during the afternoon of July 22, but by the next morning they had recovered their turgidity; however, they were severely wilted in the afternoon. On the morning of July 24 the leaves had only partially recovered, and at 1:15 p. m. one of them showed a brown necrotic wedge along the edge of the leaf cup. The apparatus was immediately stopped, the test plants were watered, and a new determination was started. However, before the second determination was under way one of the test leaves had become brown all over, as though it had been burned by fire, and it was necessary to substitute for it an adjacent leaf on the same plant.

The color change in the leaf took place in less than 10 minutes after the first browning was noted. The young leaves and the tips and margins of some of the older leaves showed the brown necrotic areas, but none of them was completely killed. The atmospheric conditions during the afternoon of this day were favorable for high rates of evaporation, since the humidity was low, the temperature high, and the sunshine bright.

During the period immediately after the watering, photosynthesis did not increase but transpiration increased somewhat. Within the next 6 days transpiration gradually regained its original rate, but 10 days after the plants were watered photosynthesis was only about two-thirds of normal.

Beginning August 8, the sand in the test plot was again allowed to dry out. Since one new test leaf was used, the expected rates of transpiration and photosynthesis were recalculated from data obtained with this leaf. The first effects of the decrease in moisture were observed on the afternoon of August 13, when the sand moisture was 1.8 percent (0.8 above the wilting point) and photosynthesis and transpiration rates were reduced by about one-third. The young leaves had wilted, but by the next morning they were again turgid. Photosynthesis and transpiration determinations for the morning of August 14 were less than one-half of the expected rates, and in the afternoon both processes were further reduced to about one-fourth of normal. The young leaves were severely wilted during the afternoon, but the test leaves, which were mature, showed no signs of wilting.

Photosynthesis and transpiration showed further reduction on the morning of August 15, when the sand moisture was 0.4 below the wilting point and the young leaves of the test plant had not regained turgidity overnight. The plants were watered at 1 p. m., and determinations for the period following showed that carbon dioxide assimilation had almost ceased and that the transpiration rate was only one-fifth of normal. By the following morning photosynthesis had markedly increased. During the next several days both processes tended to attain normalcy, but the photosynthetic activity during the afternoon period remained at about 80 percent of the expected rate until September 4 and 5, when it almost attained its original relation to the controls.

EFFECTS OF FLOODING

The data on the effects of flooding the roots of pecan seedlings growing in sand are presented in figure 2.

At 3 p. m. on July 19, after a calibration period of 5 days, excess water was added to the pot containing the test plants. The first apparent effect of the flooding became evident on July 23 when transpiration increased. On July 24 the transpiration rate continued above normal, while photosynthetic activity was about one-third below the rate expected. During the next few days photosynthesis was further reduced to approximately one-half of normal and more or less maintained that level until August 11, after which it gradually declined until August 24, 1 day after the excess water was removed, when the rate of carbon dioxide assimilation was reduced to approximately 37 percent and 18 percent of normal in the morning and afternoon, respectively.

The transpiration rate, which on July 23 and 24 was above normal, decreased rapidly during July 25 and 26. However, by July 27 transpiration had increased sharply and for the afternoon periods the rate was about normal and remained at that level until August 3; for the morning periods the rate was about 6 percent below normal and fluctuated around that level until July 31, then decreased rapidly until August 3. No determinations were made from August 3 to 8, but from the latter date to August 24 transpiration showed approximately the same degree of reduction as photosynthesis; the rates of both processes were about the same for the morning periods, but for the afternoon periods those of photosynthesis were much lower.
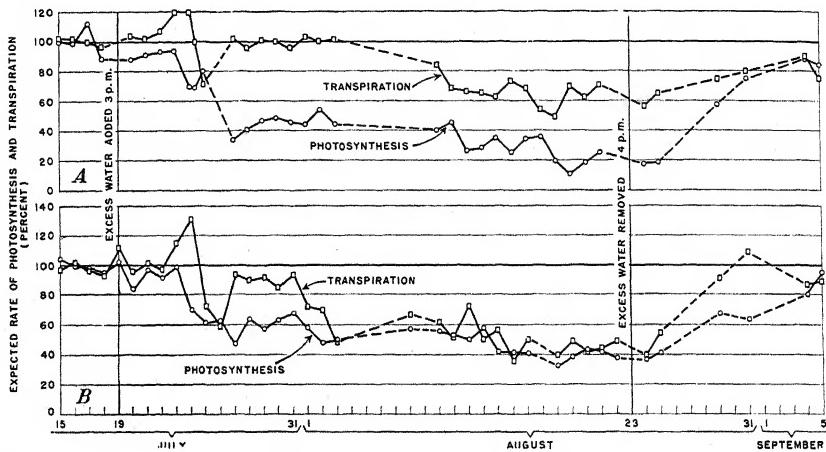

FIGURE 2.—Effect of flooding the roots on photosynthesis and transpiration of leaves of pecan seedlings growing in sand, expressed as percentage of expected rate: *A*, Afternoon determinations; *B*, morning determinations.

At 4 p. m. on August 23, the sand in the pot of flooded plants was drained and one-half of the plants of each of the three treatments were harvested for analysis. The fibrous roots of the flooded plants were black and dead, and many of the larger roots had died back as much as one-third to three-fourths of their length. Roots on the check and drought plants were all alive, and the color was normal. Figure 3, *A–C*, shows the root system of a representative plant from each treatment.

On August 24, the day following drainage of the flooded sand, there was no evidence that either photosynthesis or transpiration had begun to recover from the effects of the excess soil water, but on August 25 a slight recovery was indicated in both processes. Determinations on August 29, 6 days after the excess water had been drained off, showed a marked recovery in both processes, and by August 31 and September 4 and 5 both processes had attained average rates not far below those established before the plants were flooded.

About 2 weeks after the plants were flooded it was noted that the lenticels on the submerged portions of the stems had begun to enlarge and appeared white and spongy. At the time the excess water was drained off and the first plants were harvested for analysis, the lenticels had made considerable development. Figure 3, *D–F*, shows
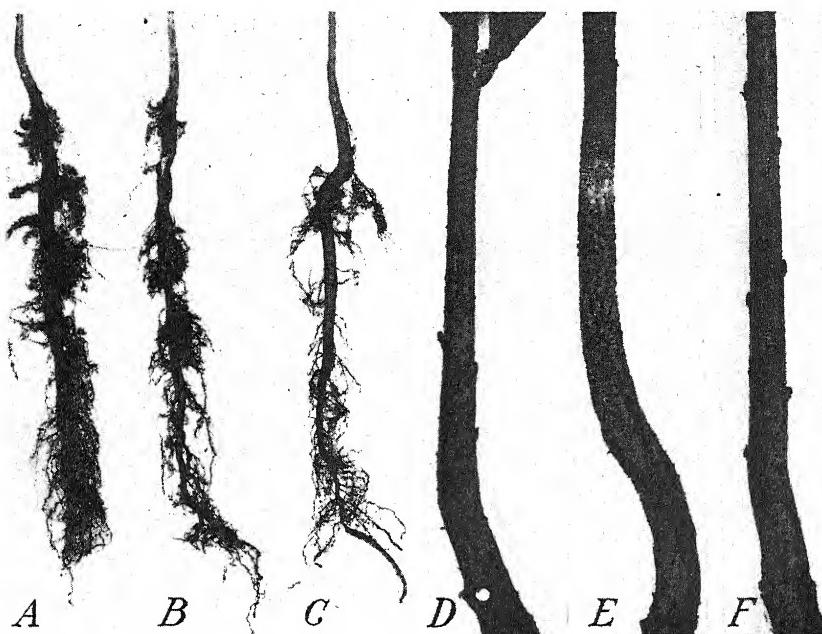
FIGURE 3.—*A–C*, Root systems of representative pecan seedlings grown in sand: *A*, Check plant; *B*, flooded plant; *C*, drought plant. Note sparse root system on drought plant.   Most of the fibrous roots on the flooded plants are blackened and dead, and the taproot and many laterals are partly dead.   *D–F*, Stems of representative pecan seedlings grown in sand: *D*, Check plant; *E*, flooded plant; *F*, drought plant.   Note lenticular proliferation on stem of flooded plant.

the lenticels on a typical flooded plant compared with those on typical check and drought plants.

The leaves of flooded plants began to lose some of their green color about 3 weeks after they were flooded and soon became mottled in appearance. The mottling, which resembled symptoms characteristic of certain mineral deficiencies, became more pronounced during the period when the excess moisture was maintained, but within a week after the soil was drained the mottling began to disappear.

EFFECTS OF FLOOD AND DROUGHT CONDITIONS ON COMPOSITION OF PECAN SEEDLINGS

The data from the analysis of the plants harvested on August 23, at the time the flooded plants were drained (table 1), showed a marked difference in the percentages of nitrogen in the tissues of check and flooded plants (dry-weight basis). The percentage of nitrogen was 0.45 higher in check plants (stems and roots) and 0.69 higher in the leaves than in similar tissues of plants grown for 35 days in flooded sand. The tissues of plants subjected to drought conditions contained slightly lower percentages of nitrogen than those of the check plants. Analysis of the plants harvested September 18 (26 days after the excess water was drained from the sand) showed an increase of 0.1 in percentage of organic nitrogen in the leaves of both check and drought plants and of 0.38 in the leaves of flooded plants, but the leaves of the

check plants still contained a higher percentage of nitrogen than leaves of the flooded plants. The roots and stems of the check and drought plants showed no appreciable change in the percentages of nitrogen as compared with plants of similar treatments harvested August 23, but the roots and stems of the flooded plants showed a gain of 0.22 in nitrogen percentage.

TABLE 1.—*Organic nitrogen and ash in pecan seedlings grown in sand under different moisture conditions*

Lot No.	Treatment	Tissue	Date	Organic nitrogen (dry-weight basis)	Ash (dry-weight basis)
				Percent	*Percent*
1	Check	Roots and stems	Aug. 23	1.32	5.43
			Sept. 18	1.30	5.61
		Leaves	Aug. 23	2.49	9.60
			Sept. 18	2.59	9.88
2	Drought	Roots and stems	Aug. 23 [1]	1.23	5.25
			Sept. 18 [2]	1.25	5.63
		Leaves	Aug. 23 [1]	2.30	8.99
			Sept. 18 [2]	2.42	9.08
3	Flooding	Roots and stems	Aug. 23 [1]	.87	4.38
			Sept. 18 [2]	1.09	5.59
		Leaves	Aug. 23 [1]	1.80	7.58
			Sept. 18 [2]	2.18	8.19

[1] 35 days after lot 3 was flooded and 8 days after second drought was terminated in lot 2.
[2] 26 days after excess water was drained from lot 3 and 34 days after second drought was terminated in lot 2.

Analysis of plants harvested August 23 showed lower percentages of ash in all tissues of the flooded plants than in those of the check and drought plants. However, in the plants harvested September 18 the percentage of ash in the roots and stems of flooded plants had increased so that it was about the same as that in similar tissues of the check and drought plants. The leaves of the flooded plants also showed a marked gain in the percentage of ash from August 23 to September 18, but they were still 1.69 below the check leaves in percentage of ash.

SEEDLINGS GROWING IN SOIL

EFFECTS OF DROUGHT

The data on the effects of drought on pecan seedlings growing in soil are presented in figure 4.

During the calibration period (August 26 to September 7) all seedlings received ample moisture, but the test plants were not watered from September 7 to 23. During this time the soil moisture in the test pot gradually dried out until it was slightly below the wilting point.

The first effects of the drying were apparent on the afternoon of September 16, when the soil moisture dropped to 18 percent. At the same time there were small decreases in both photosynthesis and transpiration, and these continued to decrease at about the same rate during the afternoons of September 17 and 18.

The data for the morning determinations show no consistent deviations until September 21, when the transpiration rate dropped to one-half of normal. Photosynthesis was slightly depressed during the
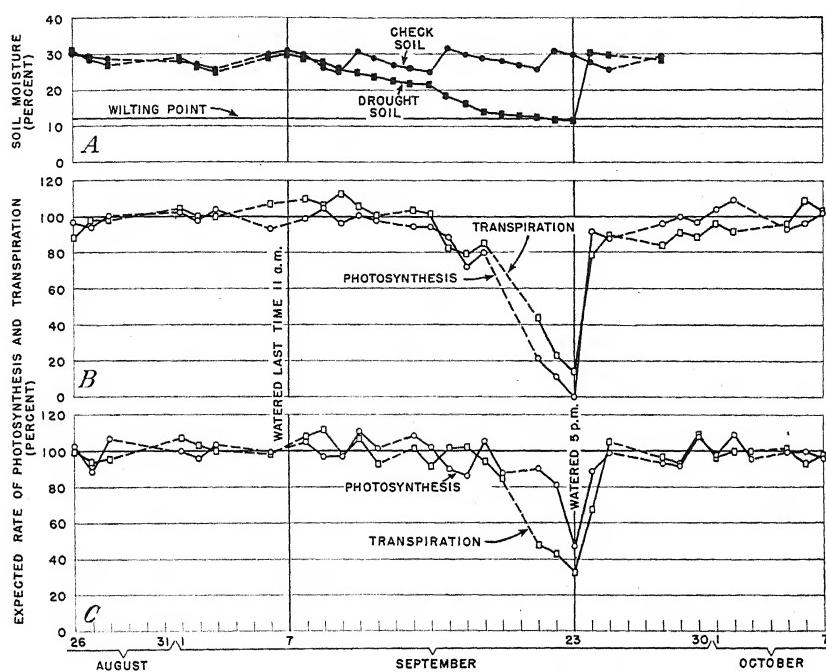
FIGURE 4.—Effect of drought on photosynthesis and transpiration of leaves of pecan seedlings growing in soil: *A*, Moisture percentages in soil; *B* and *C*, photosynthesis and transpiration (afternoon and morning determinations, respectively) expressed as percentage of expected rate.

morning of that day, but by afternoon it was reduced to one-fifth of the expected rate, while the transpiration rate was about the same as that of the morning period. The next morning the soil moisture had reached the wilting point and both processes were depressed somewhat further than on the previous morning; in the afternoon photosynthesis was further reduced to one-tenth and transpiration to one-fourth of the expected rates. During the morning of September 23 the soil moisture was below the wilting point and the rate of carbon dioxide assimilation was reduced to one-half of normal while transpiration was about one-third of the expected rate. By afternoon photosynthesis had ceased and there was more carbon dioxide in the air after it passed over the test leaves than before, indicating that the respiration rate was proportionately greater than that of assimilation. The leaves, which were mature, showed no signs of wilting; similar leaves of plants subjected to drought in sand did not wilt either.

The test plants were watered at 5 p. m., and on the morning of the following day (September 24) a marked recovery was noted in both photosynthetic activity (89 percent of normal) and in the transpiration rate (67 percent of normal); in the afternoon carbon dioxide assimilation had recovered to 92 percent and transpiration to 79 percent of the expected rates. Within the next 5 days both processes had attained approximately normal rates.

EFFECTS OF FLOODING

With a few notable exceptions, the effects of flooding on seedlings grown in soil (fig. 5) were similar to those on seedlings grown in sand.

The pretreatment relation was established from August 26 to September 2, and the test plants were flooded at 8 a. m. on September 3. The first apparent effects of flooding were evident on the morning of September 8, when the rate of carbon dioxide assimilation was reduced by 21 percent; but in the afternoon there was no appreciable effect on the process. Subsequent determinations for morning and afternoon showed a steady decline in the rate of photosynthesis until September 23, when the excess water was removed. The amount of depression was considerably more severe in the afternoons than in the mornings, and the process had ceased by the afternoon of September 23.
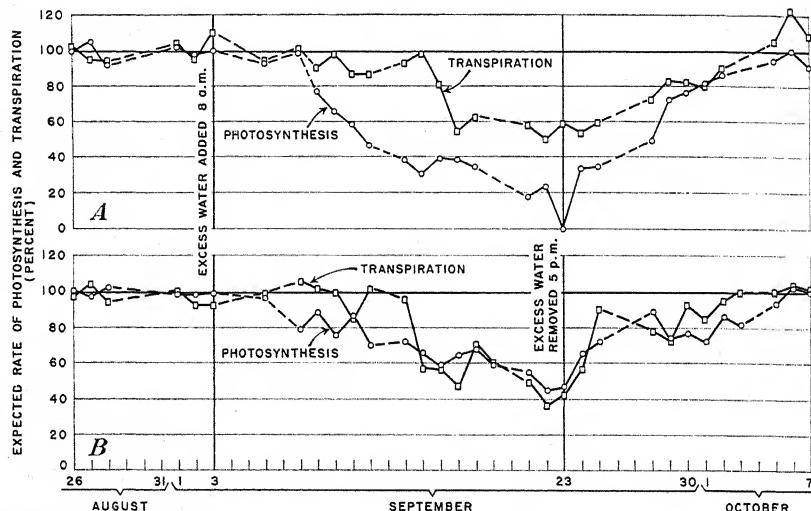

FIGURE 5.—Effect of flooding the roots on photosynthesis and transpiration of leaves of pecan seedlings growing in soil, expressed as percentage of expected rate: *A*, Afternoon determinations; *B*, morning determinations.

Although transpiration was generally somewhat depressed, there was no consistent reduction until the morning of September 15, 12 days after the plants were flooded. From this time until the soil was drained on September 23, transpiration declined gradually until it was about one-half of normal. The data for the morning of September 24, the day after drainage of the soil, show some recovery in both processes, and subsequent determinations show a gradual recovery to normal by October 7.

DISCUSSION

It is evident from the data presented that either deficient or excessive amounts of soil moisture resulted in subnormal rates of photosynthesis and transpiration in the leaves of pecan seedlings. The degree of depression of the rates depended, among other things, upon

the severity and duration of the adverse soil-moisture conditions and the atmospheric conditions prevailing at the time.

The direct cause of the depression of photosynthesis and transpiration in leaves of drought plants is undoubtedly a water deficit in the leaves. Although no stomatal observations were made, it is not unlikely that complete or partial closure of the stomata, which is usually correlated with loss of turgidity in the leaf cells, was a factor in the reduced photosynthesis rate under conditions of marked moisture deficiency in the leaves. There were no signs of wilting of the mature leaves, but there is little doubt that they suffered from a water deficit when the soil moisture was at or near the wilting point. The leaves usually overcame the water deficit to some extent during the nights, and because of the relatively cool temperature, high humidity, and reduced light conditions during the mornings, there were no appreciable effects on photosynthesis and transpiration until the moisture in the sand or soil approached the wilting point. On the other hand, both processes were reduced considerably in the afternoons before the moisture in the sand or soil had closely approached the wilting point.

It is natural to expect the depressions to be greater during the afternoons than during the mornings, because the vapor pressure gradients between the leaves and atmosphere are greater; such conditions probably would accentuate the effects of the drought in the afternoons, since it is unlikely that the root systems could absorb the small amounts of available water in the drought soils as fast as it could be removed by transpiration. During the morning periods, however, the leaf cells were probably more turgid and the temperature, light, and humidity conditions were less favorable for evaporation; as a result, transpiration and photosynthesis rates probably would be depressed less. In this connection it is interesting to note that for several days before the moisture in the heavy soil reached the wilting point the rates of photosynthesis and transpiration of the leaves were considerably depressed during the afternoon periods but were affected only slightly, if at all, during the mornings. For plants in sand this condition prevailed for only 1 day, and the difference in behavior was probably due largely to differences in the rates at which the moisture decreased in the two soil media.

Taylor and Furr (*12*) emphasized the distinction between the first wilting percentage (the soil moisture at which wilting occurs but at which plants will recover if placed in a saturated atmosphere) and the ultimate wilting percentage (the soil moisture at which the leaves will not recover turgidity even when in a completely saturated atmosphere). It seems probable that the plants growing in soil were in this so-called wilting range when they showed recovery in the morning but serious curtailment of photosynthesis and transpiration during the afternoon.

That the amount of injury caused by drought is largely dependent upon the prevailing weather conditions during the time that the soil moisture is at or near the wilting point, was indicated by the behavior of leaves during the three drought periods. In experiment 1, during the first drought period, the moisture in the sand was allowed to go below the wilting point on 1 day only, but the leaves suffered severe damage in a short time during the afternoon of that

day because of rapid evaporation due to high temperature and low humidity. In less than half an hour many of the leaves were visibly injured as a result of desiccation. During the second drought period, however, the atmospheric conditions were much less favorable for evaporation and there was no apparent damage to the leaves. Likewise, in experiment 2, with seedlings growing in heavy soil, the soil moisture was at or below the wilting point for 3 days during which the humidity was high, the weather was partly cloudy and relatively cool, and the leaves were not visibly injured. There was a marked depression in rates of carbon dioxide assimilation and transpiration in leaves of plants growing in both sand and heavy soil during the drought period, but in all cases there was also a marked recovery in the rates of both processes on the day after watering, which indicates that most of the leaf cells were not permanently injured by the water shortage.

The data obtained in the drought studies, insofar as they are applicable to field conditions, give emphasis to the importance, in semiarid regions or in orchards where irrigation is practiced, of maintaining the soil moisture at a level appreciably above the wilting point, particularly during the hot, dry months, when drought may impair the normal functioning of the leaves or cause permanent damage to the foliage and nuts and ultimately injure the entire tree. Furthermore, many of the young fibrous roots are killed during a period of drought, and as a result subsequent mineral deficiencies may occur in the tree before new rootlets can be formed after the drought is terminated.

The deleterious effects of flooding on photosynthesis and transpiration are undoubtedly due to depletion of the oxygen supply to the roots, causing abnormal root activity, such as a reduced rate of respiration, subnormal absorption of minerals and water, and eventually severe damage to the root system. The facts that the dry-weight percentages of organic nitrogen and ash in the tissues decreased during the period of flooding but increased rapidly after the sand was drained and that the mineral-deficiency symptoms which appeared in the leaves of flooded plants cleared up soon after the sand was drained indicate that flood conditions caused subnormal absorption of minerals by the plants.

SUMMARY

The effects of both excessive and inadequate amounts of soil moisture on photosynthesis and transpiration of pecan leaves were determined on pecan seedlings growing in coarse sand and in heavy soil.

In all instances, both soil-moisture extremes caused subnormal rates of photosynthesis and transpiration in the leaves, the degree of reduction depending primarily upon the severity and duration of the adverse soil-moisture conditions and on the atmospheric conditions during the critical periods.

Under drought conditions a marked reduction in the rates of both photosynthesis and transpiration occurred 1 or 2 days before the moisture in the sand or soil had reached the wilting point. Transpira-

tion and photosynthesis rates were usually depressed at about the same time, and as a rule the rates of both processes were depressed in the afternoon periods 1 or 2 days before any appreciable reduction was observed in the mornings, probably owing largely to wide differences in atmospheric conditions that prevailed during the afternoon and morning periods.

The amounts of reduction in the rates of [photosynthesis 'and transpiration of leaves of pecan seedlings subjected to drought were closely correlated with the proximity of the soil moisture to the wilting point as well as with the atmospheric conditions during the critical periods of moisture shortage. Under conditions highly favorable for moisture evaporation, as in the afternoons, photosynthesis almost ceased when the soil moisture was at the wilting point or slightly below; but the reduction in transpiration was considerably less. Under conditions less favorable for evaporation, as in the mornings, the transpiration rates were reduced to a greater extent relative to the reduction in the rates of photosynthesis, although the actual reduction in transpiration was usually somewhat less than that under conditions highly favorable for evaporation.

The rate of recovery in photosynthesis and transpiration activity from the effects of drought [was usually very rapid during the first day or two after termination of the drought, but several days more were required before the rates reached normal or their maximum.

A substantial reduction in the rate of photosynthesis in leaves of pecan seedlings with submerged roots was observed 5 days after submersion, but no consistent and definite depression of transpiration occurred until several days later. Photosynthesis was depressed to a greater degree in the afternoons than in the mornings, as was the case in plants under drought conditions. The photosynthesis rate of leaves of flooded plants in sand was reduced to a low of 11 percent of normal, while in leaves of flooded plants in soil it was reduced to cessation, thus indicating that the supply of oxygen to the roots was reduced to a greater degree in the latter medium.

The percentages of organic nitrogen and ash in tissues of seedlings subjected to flood conditions in sand for 35 days were considerably lower than in similar tissues of check plants. However, on the 26th day after the sand was drained the percentages of the constituents had increased considerably in relation to those for check plants but still remained lower, except that the percentages of ash in stems and roots were then about the same in the flooded and check plants. The percentages of organic nitrogen and ash were usually slightly lower in drought plants than in the check plants.

The results of these experiments emphasize the importance of good internal and surface drainage in pecan soils, in addition to an adequate moisture supply and a high moisture-holding capacity, and suggest possible causes of crop failures in orchards or groves in which the soils are subjected to drought or flood or to alternations of the two conditions.

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INTERACTION OF SEX, SHAPE, AND WEIGHT GENES IN WATERMELON [1]

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INTRODUCTION

Several workers on inheritance in watermelon (*Citrullus vulgaris* Schrad.) have independently reported relation between fruit shape and sex and between fruit shape and weight. The reported relation between shape and weight, if consistently true, will have some economic importance in view of the present demand for smaller high-quality watermelons. Weetman (*13*)[2] found that shape inheritance when represented quantitatively by an index computed by dividing equatorial by polar diameter may be classified qualitatively to show a phenotypic relation of one pair of alleles, round vs. elongate, with F_1 phenotype intermediate and F_2 segregating into 1 round to 2 intermediate to 1 elongate. The same author found a highly significant negative correlation of low magnitude between fruit shape and weight, suggesting that long fruits are heavier than round ones. Rosa (*9*) reported that the monoecious sex habit was completely dominant over the andromonoecious by one pair of alleles and that andromonoecious plants produced fruits that were more nearly round.

F_2 populations classified for weight frequently show positive skewness such as is found in geometric progressions. Many reported investigations of this kind are reviewed by Powers (*8*), MacArthur and Butler (*4*), and Charles and Smith (*2*). Powers (*8*) has developed formulas to calculate the genetic and environmental variances for segregating populations from parental and F_1 variances. Thus far little attention seems to have been given the possibility of estimating the number of genes involved in such segregations. The ideal analysis is to separate weight into its component parts, as Powers (*6, 7*) and Sinnott (*10*) have done in comparatively simple cases. With complete information, however, any given case may disclose some groups of genes operating with geometric effects and others with arithmetic effects.

Student (*12*) presented a gene analysis of Winter's data (*14*) on the results of mass selection for high and low oil content in an open-pollinated maize variety. By using a genetic standard deviation derived from the given gross uncorrected deviations for 28 years, Student estimated that the number of genes segregating for oil content was probably of the order 20 to 40.

Goodale (*3*) studied weight in the albino mouse after positive selection for about 7 years and estimated that at least 32 genes were segregating. Butler (*1*) studied ovary weight in the tomato and estimated the segregation of 6 genes because he was able to obtain a good-fitting binomial with the exponent 6. Goodale and Butler used the uncor-

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rected gross measurements, but Student based his estimate of gene number upon a genetic classification. It is shown in the present investigation, however, that a good-fitting binomial can be found for any postulated exponent provided the correct geometric mean for the observed population has been estimated.

The fact that a quantitatively measured character like watermelon fruit shape is controlled by one pair of genes has been used in estimating the number of genes segregating in a more complex character, such as fruit weight. The present authors have studied several crosses in which genes for sex, shape, and weight are segregating in the same populations.

MATERIALS AND METHODS

At the United States Regional Vegetable Breeding Laboratory, Charleston, S. C., a number of crosses have been made between round, light-weight, andromonoecious and long, heavy-weight, monoecious varieties of watermelon, viz, Northern Sweet $\times$ Dove, Baby Delight $\times$ Dove, Baby Delight $\times$ Peerless, and Winter Queen $\times$ Sun Moon and Stars. The first and third crosses were carried to F_3 and backcrossed to 1 or both parents. In the F_3 of Baby Delight $\times$ Peerless complete classification was made for shape only. In the cross of Northern Sweet $\times$ Dove all 3 characters were studied simultaneously in each of the 2 parents, F_1 generation, and F_2. In this experiment there were 10 replicated plots each of which contained 3 rows of F_2 and 1 row of the Northern Sweet parent. In addition, 2 rows of F_1 and 1 row of Dove were included at random to furnish data on all generations involved.

The seed was planted in flats in the greenhouse, and individual plants were transferred to pots before being transplanted to the field. In all cases the rows were 12 feet apart and the hills were 3 feet apart in the row. The vines were pruned to one runner during the first 3 weeks of growth. Consequently, fruit weights in these experiments are not comparable with those of commercial plantings, but all data within the experiments are comparable.

The Dove variety used in these investigations is known commercially under many names, including Gandy, Garrison, Dude Creek, Dude Ranch, Coker, Old Darlington, and Darlington County. It has a history of originating from a few seeds transported in 1865 from Chattanooga, Tenn., to Darlington County, S. C., by J. C. Dove, of Dovesville, S. C. In 1937, when these crosses were made, it had not been inbred; consequently the F_1 plants of all these crosses were segregating for some characters, including weight but not shape. Dove is one of the best quality watermelons tested at the Vegetable Breeding Laboratory. Early recognition of this fact accounts for the crosses that were made before self-pollinated lines were available.

Of the three characters comprising this study, two (weight and shape) are measured quantitatively but the third (sex habit) can be classified only qualitatively. Shape measurements can readily be converted from quantitative into qualitative data, and this has recently been done in watermelon populations segregating for two pairs of genes for seed length (5). Comparison of observed and calculated Mendelian ratios was made for the two qualitatively classified characters with the use of gene symbols as follows: A monoecious and a andromonoecious sex habit; O elongate (long) and o spherical shape of

fruit (*Oo* semilong is distinguishable in some crosses). *A* vs. *a* classification was made at floral anthesis, and *O* vs. *o* was made after calculation of the shape index. The construction of frequency diagrams revealed the most probable division points separating the shape phenotypes.

Frequency histograms were made with class intervals for the shape and weight range based on one-third of the standard deviation. The magnitude of the genetic class interval used for regrouping the original shape measurements in F_2 populations was obtained by geometric expansion [3] of the binomial $(x + y)^2$ where x and y represent the mean shape indices of the two parents.[4] If the 1 to 2 to 1 ratio is treated geometrically there is a phenotypic overlap between the long and semilong groups producing the observed ratio 3 long to 1 round found in F_2 or F_3 of the Northern Sweet $\times$ Dove cross.

Practice with the expansion of binomials in 3 years of investigating complex quantitative characters such as weight and sugar content has given rise to ways of estimating the number of segregating genes. The genetic standard deviation (S_g) [5] is calculated from the original field data of segregating and homozygous parental populations as

$$S_g = \sqrt{S_m^2 - S_e^2}$$

where

$S_m^2 =$ variance of segregating population

$S_e^2 =$ variance from environmental sources

The environmental variance (S_e^2) for the cross of Northern Sweet $\times$ Dove was estimated by using only the variance of the Northern Sweet parent.

To analyze the monofactorial shape inheritance in the 490 F_2 fruits of the observed population of Northern Sweet $\times$ Dove the indicated binomial is $(0.5 + 0.9)^2$, in which the two terms are the approximate mean shape indices of the two parents. When the binomial standard deviation (S'_g) is calculated and adjusted for frequency disparity [6] the two standard deviations become 0.145 for the observed population S_g and 0.142 for the binomial S'_g. That is, the F_2 variance from total sources (see table 2) is 0.0228, the S_e^2 of the Northern Sweet fruits is 0.0017; hence the genetic standard deviation is

$$S_g = \sqrt{0.0228 - 0.0017} = 0.145.$$

General problems in quantitative inheritance, however, cannot be assumed to be controlled by one pair of factors; but the generalized binomial $[1 + (n + 1)]^n$ may be used for any assumed number of

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[3] Thus, the geometric expansion of $(1 + 32)$ is $1 (1) + 5 (2) + 10 (4) + 10 (8) + 5 (16) + 1 (32)$, in which the binomial coefficients are found by the ordinary rule for expanding a binomial to the 5th power and the several term values are found by multiplying the value of the first term of the series and each successive value by the common ratio, viz, $\sqrt[5]{32/1} = 2$.

[4] This would not be correct if transgressive variation had been present.

[5] The authors are indebted to Prof. W. G. Cochran of Iowa State College for furnishing the method of correcting the original data for environmental effect and for directing their attention to the formula for the genetic standard deviation.

[6] The sum of the coefficients for a binomial raised to the 2d power is 4; adjustment of a frequency of 4 to 490, the observed population frequency, was made by multiplying the sums and sums of squares for the generalized binomial by 490/4, or 122.5.

factors, and for one pair it would be $(1+3)^2$. [7] The standard deviation of this binomial expanded by the rules in footnote 3 is 0.831, which when adjusted for frequency becomes 0.721 (S_{bi}). These two binomials, $(0.5+0.9)^2$ with a unit spread of 0.4 and $(1+3)^2$ with a unit spread of 2, are in direct proportion to their standard deviations, that is, 0.4 is to 2 as 0.142 is to 0.710.

The adjusted binomial standard deviation of 0.710 (S'_g) is almost identical with the adjusted standard deviation of the generalized binomial of 0.721 (S_{bi}). Therefore, in general problems of quantitative inheritance estimation of gene number can be made from tables showing values of the adjusted standard deviation of the generalized binomial, S_{bi}, [8] as given later (p. 545.).

A formula [9] for estimating S_{bi} when exponents are too high for calculation by machine or ordinary use of logarithms follows:

$$S_{bi}=\left[\frac{(1+r^2)^n}{(2)}-\frac{(1+r)^{2n}}{(2)}\right]^{\frac{1}{2}}$$

$$2n=\Sigma f$$

$$(1+r)^n=\Sigma f_v$$

$$(1+r)^{2n}=\Sigma f_v^2$$

where

n=number of genes or binomial exponent

$r=\sqrt[n]{(1+n)}$

f=population frequency

v=class value

In adjusting S_{bi} values for frequency, f, f_v, and f_v^2 terms are multiplied by the ratio of the population frequency to the generalized binomial frequency.

The shape and weight data of F_2 and backcross populations were corrected for percentage departure from the general mean of the population if the variance due to blocks had a significant F value. The variance and covariance between shape and weight in the F_2 of Northern Sweet $\times$ Dove were calculated in two ways: (1) By using the observed frequencies and values; (2) by using expected subclass sums according to a method developed by Snedecor and Cox (*11*). The adjusted sums, but not the adjusted numbers, are useful in calculating variance when Mendelian phenotypes are included in a variance analysis.

The number of genes segregating for weight in 490 F_2 plants of the population of Northern Sweet $\times$ Dove was estimated by an extension of the foregoing formulas.

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[7] Because standard errors of binomials in the series $[1+(n+1)]^n$, where values of n represent all possible numbers of genes, differ according to differences in gene number.

[8] S_{bi} for n number of segregating genes are given in column 4 of table 6 and values from 2 to 30 genes adjusted for a frequency of 490 are given in column 6 of table 5.

[9] Made by Dr. L. A. Dye of the Mathematics Department of The Citadel, Charleston, S. C.

ANALYSIS OF EXPERIMENTAL RESULTS

FRUIT SHAPE IN F₂ AND BACKCROSS POPULATIONS

Figure 1 shows frequency histograms for distributions of fruit shapes in three series of crosses involving round and long parents and backcrosses to one or both parents. When distribution is made on intervals of one-third of the standard deviation, a discontinuous multimodal grouping is shown. The grouping permits qualitative classifications, which are roughly distributed in backcrosses on a 1 to 1 ratio and in F₂ 3 long to 1 round in two cases and 1 long to 2 semilong to 1 round in the third case.

To the right side of each original histogram the population is shown regrouped on a genetic interval as calculated by binomial expansion. The χ^2 values for the theoretical and observed ratios of 1 long to 2 semilong to 1 round show excellent fits, even in the cases of the two F₂ populations giving 3 long to 1 round, in a way to suggest a 1 to 2 to 1 segregation with geometric rather than arithmetic interaction.
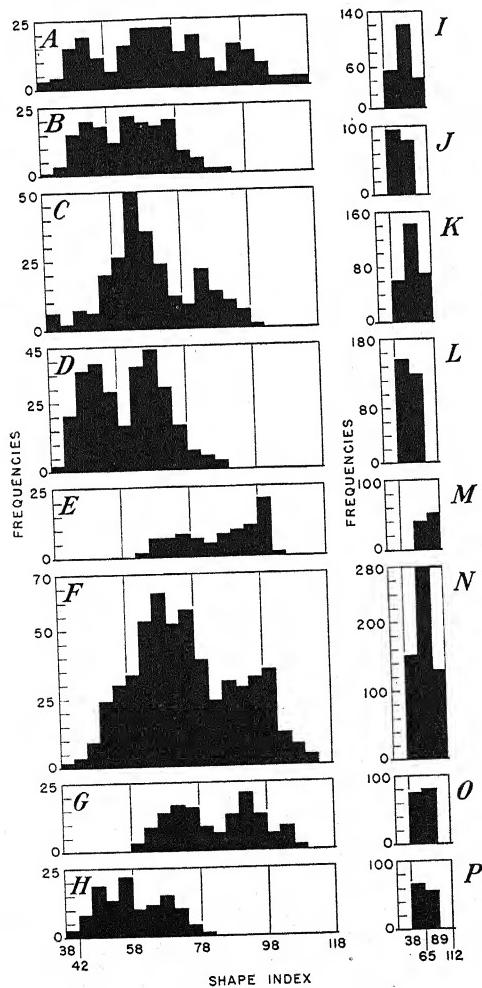

FIGURE 1.—Frequency histograms for distributions of fruit shapes in crosses involving watermelon parents producing long and round fruits. *A–H*, Frequencies grouped on intervals of one-third standard deviations. *I–P*, Frequencies grouped on calculated genetic intervals. *A* and *I*, F₂ of Dove × Baby Delight; χ^2, 1.9152. *B* and *J*, (Dove × Baby Delight) × Dove; χ^2, 0.5650. *C* and *K*, F₂ of Sun Moon and Stars × Winter Queen; χ^2, 1.5056. *D* and *L*, (Sun Moon and Stars × Winter Queen) × Sun Moon and Stars; χ^2, 0.8981. *E* and *M*, (Sun Moon and Stars × Winter Queen); χ^2, 1.1494. *F* and *N*, F₂ of Northern Sweet × Dove; χ^2, 2.7211. *G* and *O*, (Northern Sweet × Dove) × Northern Sweet; χ^2, 0.0462. *H* and *P*, (Northern Sweet × Dove) × Dove; χ^2, 0.8850.

For example, with $(0.51+0.93)^{2}$ [10] the arithmetic mean is 0.72 and the geometric mean is 0.69, which is closer to the mode in figure 1 for the F_2 population of Northern Sweet $\times$ Dove.

FRUIT SHAPE IN F_3 POPULATIONS

Data on inheritance of fruit shape in two F_3 generations are shown in figure 2 by histograms for segregating and nonsegregating groups. The numerical proportions of segregating to nonsegregating, given in the histograms, are in accord with the foregoing observations for F_2. In the Peerless $\times$ Baby Delight populations the phenotypic as well as genotypic ratios more closely approximated the 1 to 2 to 1 pattern, but in the Northern Sweet $\times$ Dove populations the phenotypic ratio of segregating populations continued to show considerable overlap between the long and semilong phenotypes producing a ratio of 3 long to 1 round, although a complete genotype classification of the F_2 parents is 1 long to 2 semilong to 1 round.

INTERACTION OF SEX AND FRUIT SHAPE

When gene interaction between sex and shape was studied, as in the histograms of figure 3, a pronounced linkage was disclosed. The measured data, when analyzed for χ^2 and linkage values in table 1, indicate strong interaction values for genes A and O in all crosses. In the Northern Sweet $\times$ Dove segregating populations χ^2 for gene A is significant or highly significant in all three cases and for O in one of the three; but the two backcrosses and Dove $\times$ Baby Delight F_2 populations show no disturbance in segregation of these two pairs of alleles. In all cases, therefore, coupling linkage in segregating populations causes an association of round fruits with the andromonoecious sex habit and of long fruits with the monoecious sex habit.

TABLE 1.—*Orthogonalized χ^2 values for A, O, and ao and the calculated linkage values for various crosses and backcrosses of watermelons*

Crosses and backcrosses	Frequencies in phenotypic class					χ^2			Degrees of freedom	Linkage value
	AO	Ao	aO	ao	Total	A	O	ao		
Crosses:										
Dove $\times$ Baby Delight										
$\quad$ F_2 population	139	32	32	27	230	0.001	0	16.001*	1	0.323±0.039
Northern Sweet $\times$ Dove:										
$\quad$ Segregating F_3 populations	1,261	169	267	388	2,085	45.418**	3.178	629.521**	1	.207± .010
$\quad$ F_2 populations–1	127	35	26	46	234	3.851*	11.031**	58.167**	1	.258± .034
$\quad$ F_2 populations–2	157	34	66	44	301	20.785**	.071	22.158**	1	.350± .035
$\qquad$ Total	1,684	270	391	505	2,850					
$\quad$ Deviation χ^2						62.669**	6.193*	700.220**	1	
$\quad$ Heterogeneity χ^2						7.386	8.087*	25.627**	3	
$\qquad$ Total χ^2						70.055**	14.280**	725.847**	4	
Backcrosses:										
(Sun Moon and Stars $\times$ Winter Queen) $\times$ Winter Queen	33	7	10	35	85	.019	0	25.988**	1	.200± .043
(Peerless $\times$ Baby Delight) $\times$ Baby Delight	53	11	8	68	140	.864	2.064	72.864**	1	.136± .029
$\qquad$ Total	86	18	18	103	225					
$\quad$ Deviation χ^2						1.138	1.138	102.684**	1	
$\quad$ Heterogeneity χ^2						.255	.926	3.832	1	
$\qquad$ Total χ^2						.883	2.064	98.852**	2	

*Significant at 5-percent level; **significant at 1-percent level.

[10] The parental mean shape indices.
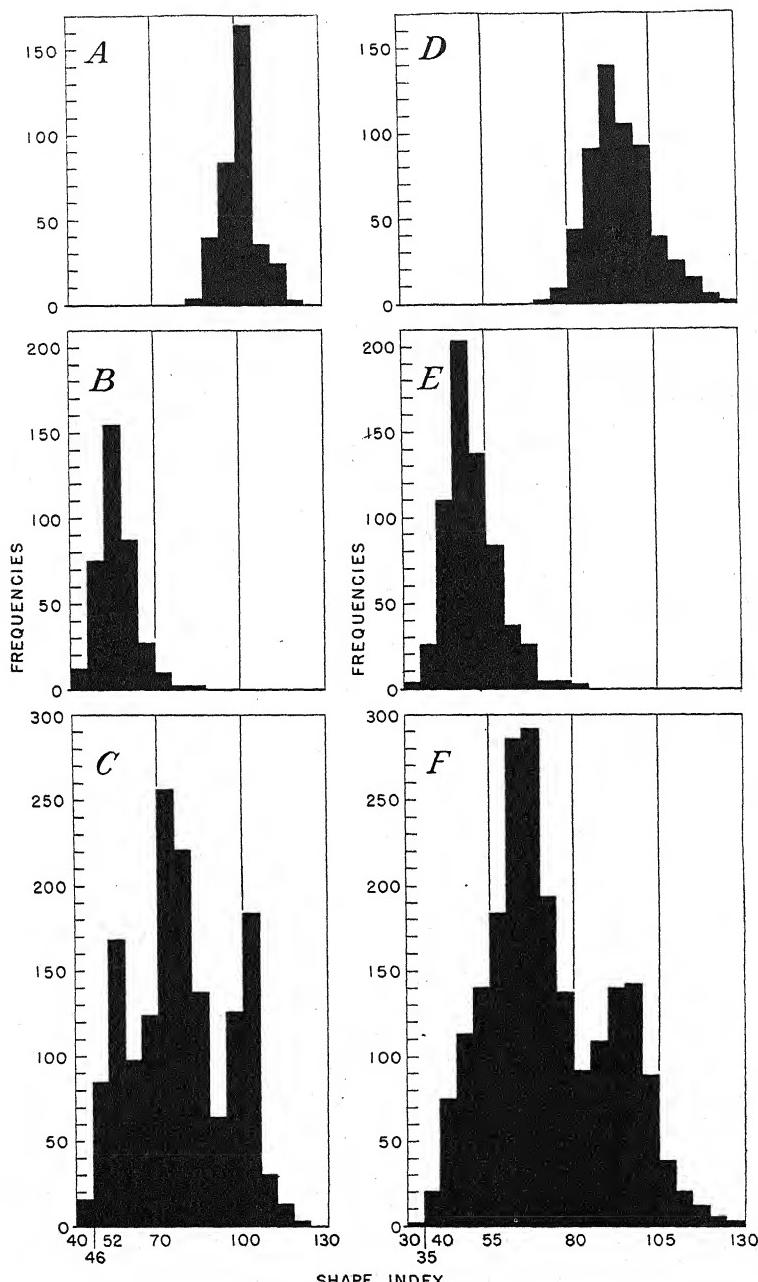
FIGURE 2.—Frequency histograms for phenotypic distributions of fruit shapes in nonsegregating and segregating F_3 populations of watermelons.   *A–C,* Peerless × Baby Delight: *A,* 8 all-round populations; *B,* 8 all-long populations; *C,* 21 segregating populations.   *D–F,* Northern Sweet × Dove: *D,* 10 all-round populations; *E,* 12 all-long populations; *F,* 31 segregating populations.

WEIGHT AS INFLUENCED BY SEX AND SHAPE

The effect of interaction of some of the weight-determining genes and the qualitatively classified allelic pairs for sex and shape is shown graphically and statistically in figure 4 and tables 2 and 3.

According to graphs and variance analyses three of the crosses show that sex genes have no effect on weight distribution but that shape genes do; in the backcross of Peerless $\times$ Baby Delight there is a weak reversal of the foregoing tendencies where monoecious fruits 5.08 ± 0.24 kg. (fig. 4, C) are significantly heavier than andromo-noecious fruits 4.42 ± 0.22 kg. (fig. 4, D). But the differences between long and round fruits in the same population (fig. 4, K and L) are not significant. Shape interaction is usually highly significant, but sex interaction is low or lacking. Long fruits in these crosses are there-fore apt to be heavier than round fruits, and differences between mean weights shown in the legend for figure 4 are of the same order of significance as the F values for variance analyses in table 3. The variance and covariance data for weight and shape (table 2) confirm
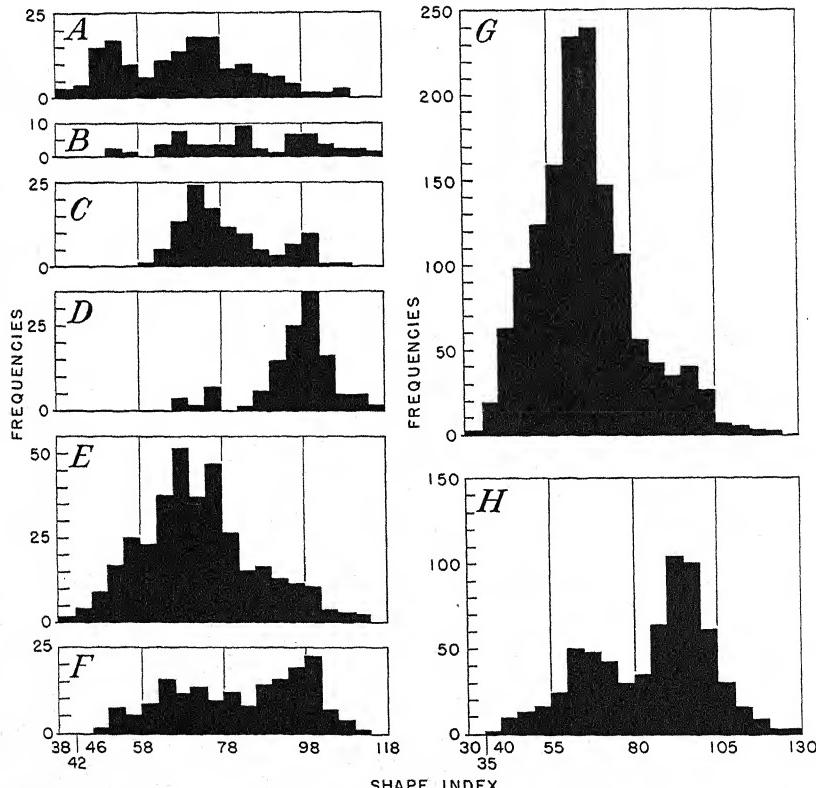
FIGURE 3.—Frequency histograms for shape of watermelon fruits in populations classified for sex habit. *A* and *B*, F_2 of Dove $\times$ Baby Delight: *A*, Monoecious; *B*, andromonoecious. *C* and *D*, (F_1 of Dove $\times$ Baby Delight) $\times$ Baby Delight: *C*, Monoecious; *D*, andromonoecious. *E* and *F*, F_2 of Northern Sweet $\times$ Dove: *E*, Monoecious; *F*, andromonoecious. *G* and *H*, F_3 segregating populations of Northern Sweet $\times$ Dove: *G*, Monoecious; *H*, andromonoecious.
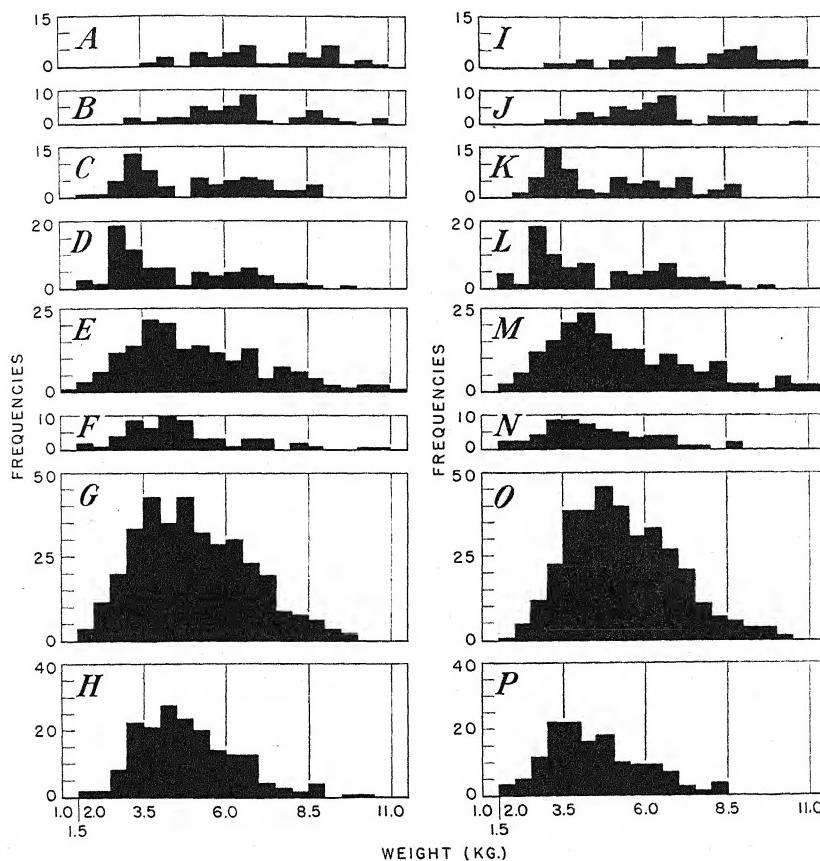
FIGURE 4.—*A* to *H*, Frequency histograms for weights of watermelon fruits in populations classified for sex habit. *A*, Monoecious (Sun Moon and Stars × Winter Queen) × Winter Queen; mean 7.41 ± 0.34 kg. *B*, Andromonoecious population of the same backcross; mean 6.94 ± 0.34 kg. *C*, Monoecious (Peerless × Baby Delight) × Baby Delight; mean 5.08 ± 0.24 kg. *D*, Andromonoecious population of the same backcross; mean 4.42 ± 0.22 kg. (difference between mean values of *C* and *D* significant at 5-percent level). *E*, Monoecious F_2 of Dove × Baby Delight; mean 5.12 ± 0.17 kg. *F*, Andromonoecious of the same cross; mean 4.70 ± 0.25 kg. *G*, Monoecious F_2 of Northern Sweet × Dove; mean 5.05 ± 0.12 kg. *H*, Andromonoecious of the same cross; mean 5.00 ± 0.09 kg. *I* to *P*, Frequency histograms for weights of watermelon fruits in the same populations as in *A* to *H*, classified for fruit shape. *I*, Long-fruited (Sun Moon and Stars × Winter Queen) × Winter Queen; mean 7.86 ± 0.31 kg. *J*, Round-fruited populations of same backcross; mean 6.46 ± 0.34 kg. (significant at 1-percent level). *K*, Long-fruited (Peerless × Baby Delight) × Baby Delight; mean 4.97 ± 0.24 kg. *L*, Round-fruited population of same backcross; mean 4.51 ± 0.22 kg. *M*, Long-fruited F_2 of Dove × Baby Delight; mean 5.25 ± 0.16 kg. *N*, Round-fruited population of the same cross; mean 4.48 ± 0.27 kg. (significant at 5-percent level). *O*, Long-fruited F_2 of Northern Sweet × Dove; mean 5.28 ± 0.09 kg. *P*, Round-fruited population of the same cross; mean 4.36 ± 0.14 kg. (significant at 1-percent level).

the data from histograms and the interpretation already given and show that the correlation between weight and shape is of the same order as observed by Weetman (*13*).

TABLE 2.—*Variance and covariance of weight and shape data from 2 sister F_2 populations of Northern Sweet×Dove watermelons*

[Variances calculated from original data and from data adjusted for expected subclass frequencies]

Sources of variation	Degrees of freedom	Mean squares for—				Unadjusted coefficient of correlation
		Weight		Shape		
		Original data	Adjusted data	Original data	Adjusted data	
Rows	29	18.0012**		0.0398		
Sex × shape	1	15.6037**	3.8756*	1.3504	0.0012	
Sex	1	.2056	14.0559**	1.0536	.0087	
Shape	1	83.0146**	106.8553**	7.4773**	7.2093**	
Sex × shape total	3	32.9413	41.5956	2.3935	2.4064	−0.8630
Error	457	2.5859	2.5490	.0054	.0060	−.3755**
Total	489	3.8258		.0228		−.3128**

*Significant at 5-percent level; **significant at 1-percent level.

TABLE 3.—*Analyses of variance of 1 F_2 and 2 backcross populations of watermelon varieties separated according to sex and shape*

Source of variation	Baby Delight × Dove			(Sun Moon and Stars × Winter Queen) × Winter Queen			(Peerless × Baby Delight) × Baby Delight		
	Degrees of freedom	Variance	F	Degrees of freedom	Variance	F	Degrees of freedom	Variance	F
Sex	1	14.078	3.32	1	4.846	1.07	1	15.268	4.13*
Shape	1	26.3541	6.22*	1	41.120	9.11**	1	7.444	2.01
Error	227	4.239		81	4.515		139	3.696	
Total	229	4.379		83	4.960		141	3.804	

*Significant at 5-percent level; **significant at 1-percent level.

Where it was possible to show the degree of correlation between shape and weight with sexes separated, the coefficient for the monoecious sex was usually higher than for the andromonoecious sex,—0.49 (326 degrees of freedom) for the former and—0.26 (160 degrees of freedom) for the latter, in F_2 of the cross Northern Sweet× Dove, comprising 490 plants. Both coefficients were highly significant.

On the other hand, there are several F_2 and backcross populations where no significant degrees of correlation are found. In Baby Delight × Dove there were coefficients of −0.13 (151 degrees of freedom) and −0.07 (53 degrees of freedom) for the two sexes; neither coefficient approached the 5-percent level of significance.

Data from F_2 and backcross populations of Dove × Baby Delight, Peerless × Baby Delight, and Sun Moon and Stars × Winter Queen also showed correlation coefficients below the 5-percent level of significance.

An improvement in the magnitude of the correlation coefficients

occurred when shape and weight data were grouped according to Mendelian phenotypes. The correlation coefficient for the Mendelian classes was -0.8630, instead of the -0.3128, as indicated in table 2 for total sources of variation.

ESTIMATING THE NUMBER OF GENES SEGREGATING FOR WEIGHT IN NORTHERN SWEET × DOVE

The magnitude of a standard deviation for weight distribution in a segregating population is governed by some other factors in addition to the environmental and genetic sources of variability already considered. Two of these are the numerical frequency of the population and the magnitude of the spread in terms of the theoretical binomial governing the population distribution. Consequently, the statistical constants for a theoretical population will require adjustment for these two factors before comparison with the statistics of an observed population is permissible.

The steps by which the number of genes segregating for weight inheritance (table 4) in the cross Northern Sweet × Dove was estimated will be first stated briefly and then illustrated.

TABLE 4.—*Constants of weight calculated from F_2 progeny and parent populations connected with the history of the cross Northern Sweet × Dove watermelons*

Type of Mendelian population	Arithmetic mean weight	Total variance (S_m^2)	Environmental variance (S_e^2)	Genetic standard deviation (S_g)
	Kg.			
Dove grown with F_2	7.46±0.85	3.633		
Dove grown with backcross to Dove	8.61± .56	4.997		
Northern Sweet grown with F_2	3.24± .08	1.446		
Northern Sweet grown with backcross of F_1 to Northern Sweet	3.30± .35	1.518		
F_1 grown with F_2	4.62± .21	1.608		
F_2	5.02	3.826	1.446	1.543
F_1 backcrossed to Dove	6.98	8.138	4.997	1.772
F_1 backcrossed to Northern Sweet	4.72	2.807	1.518	1.135

The number of genes which determine the inheritance of a quantitative character in a given population is the exponent of the generalized binomial $[1+(n+1)]^n$ whose standard deviation corresponds most closely to the value of $\bar{x}$ in the following procedure:

The value of S_g, the genetic standard deviation of an obtained segregating population, is the square root of the difference between the variance of that population (S_m^2) and the environmental variance (S_e^2) of the homozygous populations planted with it (i. e., both parents and F_1). If S_g does not fall within the limits of certain calculated S_{bi} values (see 0.707 for 1 gene and 2.310 for 100 genes in table 6), it should be transformed (by division or subtraction) until it does. S_g is then compared (see table 6) with the nearest generalized binomial standard deviation (S_{bi}), which has been adjusted for (1) differential population frequency and (2) binomial spread, as follows:

(1) Frequency: values of f, f_v, and f_v^2 in the frequency distribution for the theoretical population of S_{bi} are multiplied by the ratio of the total numbers of the observed to the theoretical populations.[11]

(2) Binomial spread: x (fully adjusted genetic standard deviation) is to S_g as the obtained population geometric mean (properly fitted and transformed with the same factor as S_g) is to the generalized binomial $[1+(n+1)]^n$ geometric mean.

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[11] Frequency adjustment for powers above 10 makes such little difference that it may be omitted.

Estimating Geometric Mean Weight

One may temporarily consider the true modal class value of the observed population as the geometric mean of the observed population. It will also be the geometric mean of the two terms of a bracketed binomial which produces a curve giving the best possible χ^2 fit with the observed population regrouped on geometrically determined class intervals. One requires a number of trial binomials all having the same geometric mean, including the values of the upper and lower limits of the segregating population range, as well as the mean values of the two parents. When one has the best fitting binomial one may calculate its standard deviation $S'_g{}^{12}$ for comparison with tabular values of S_{bi}. To illustrate:

(1) The modal class value which most nearly fits all four of the histograms belonging to the F_2 population, Northern Sweet $\times$ Dove, at the bottom of figure 4 is by inspection about 4.2 kg. or 4.4 kg., the parental weights are about 3.2 and 7.5 kg., and the weight range limits are about 1.7 and 9.7 kg. A good series of trial binomials for the two geometric means 4.2 and 4.4 are as follows:

4.2: (3.2+5.5) (2.7+6.5) (2.2+8.0) (1.7+10.4) (1.2+14.7) (0.7+25.2)
4.4: (3.2+6.1) (2.7+7.2) (2.2+8.8) (1.7+11.4) (1.2+16.1) (0.7+27.7).

(2) Geometric expansion of one or two serves to identify the most likely ones for further testing. It is necessary to obtain the frequencies and class values of a theoretical population and the class values with which to regroup the raw data for χ^2 testing. This may be done as indicated in the next section.

TABLE 5.—*Best fitting binomial terms for a geometric mean of 4.5 kg., and χ^2 values of bracketed binomials (S'_g) compared with tabular binomial standard deviations (S_{bi}) of the series $[1+(n+1)]^n$ adjusted for frequency calculated with the weight data of 490 F_2 Northern Sweet $\times$ Dove watermelon fruits*

Binomial exponent	Bracketed binomial	χ^2	5-percent probability level	Fitted or S'_g	Tabular or S_{bi} (frequency adjusted)	Difference S'_g-S_{bi} [1]
2	2.7+ 7.5	1.846	5.991	1.725	0.721	1.004
4	2.2+ 9.20	6.516	9.488	1.739	.989	.750
6	1.9+10.66	8.141	11.070	1.719	1.166	.553
8	1.7+11.9	12.967	14.067	1.688	1.291	.397
10	1.4+14.5	9.893	15.507	1.829	1.390	.439
12	1.2+16.88	10.412	14.067	1.904	1.475	.429
14	1.2+16.88	9.753	15.507	1.734	1.548	.186
16	1.0+20.25	15.568	15.507	1.874	1.592	.282
18	1.0+20.25	13.627	18.307	1.748	1.647	.101 (−0.144)
20	.8+25.31	16.353	15.507	1.937	[2] 1.699	.238 (−.092)
22	.8+25.31	17.146	18.307	1.850	[2] 1.738	.112 (−.653)
24	.8+25.31	19.799	18.307	1.738	[2] 1.777	.039 (−.014)
26	.7+28.93	23.337	18.307	1.811	[2] 1.812	.001 (+.021)
28	.7+28.93	19.955	21.026	1.734	[2] 1.845	.111 (+.054)
30	.6+33.75	15.300	18.307	1.831	[2] 1.875	.044 (+.084)

[1] Parenthetic differences were found by using 1.791 as an average value of S'_g.
[2] Higher values of S_{bi} are not frequency adjusted because binomial frequencies are so great that 10-place logarithms are inadequate to give enough significant figures and the adjusted values are increased so little that the calculation is unnecessary.

[12] S'_g values for the 490 fruits are given in table 5, column 5.

Regrouping Raw Weight Data

Each trial binomial has its own rate of difference between class values, necessitating a new regrouping of raw data for each new trial. The rate of difference for any binomial series is found by the formula: Rate equals $\left(\dfrac{\text{last term}}{\text{first term}}\right)^{\frac{1}{n}}$, where n is the exponent or any assumed number of genes. For example, in the case of $(1.2+14.7)^{14}$ the rate of difference between all terms of a geometric progression is the 14th root of 14.7/1.2, or of 12.25, readily found by logarithms. The log of 12.25 is 1.088136, and of the 14th root 0.077724, the antilog of which is 1.196. There will be 15 terms in the theoretical population of which the first is 1.2 kg.; the second and succeeding terms are obtained by multiplying by 1.196 the foregoing term value until the last term, which is 14.7, is reached. At the outset three values are known: 1.2 for the first, 4.2 for the eighth or mode, and 14.7 for the last or 15th, as convenient checks on arithmetic while multiplying. Upon learning all 15 class values, class limits are set up as follows:

Mean class values	1.2	1.4	1.7	2.1	2.5	2.9 etc.
Class limits		1.3–1.5	1.6–1.9	2.0–2.3	2.4–2.7	2.8–3.2

Regrouping the raw data into these intervals furnishes an observed frequency distribution for a χ^2 comparison with the theoretical population having the same class values, but with frequencies found as described in the following step.

Calculating Theoretical Class Frequencies

The regrouped population can be compared with the theoretical population obtained by expanding $(1.2+14.7)^{14}$ and adjusting the coefficients of each term for the obtained total of 490. The theoretical population total of the 14th power is 16,384 (table 6); therefore it is necessary to multiply the coefficient for each term by 490/16,384, or 0.0299.

Class values [1]	1.4	1.7	2.1	2.5	2.9	3.5
Observed [2]	1	6	3	21	40	58
Calculated [2]	.4	2.7	10.9	30.0	59.9	89.8

Class values [1]	4.2	5.0	6.0	7.2	8.6	10.3	12.3
Observed [2]	111	79	85	56	24	5	1
Calculated [2]	102.6	89.8	59.9	30.0	10.9	2.7	.4

[1] Class values above and below the range of observed values are omitted.

[2] Total frequency is 490.

TABLE 6.—*Data for estimating the probable number of Mendelian genes segregating in quantitative inheritance problems with geometric interaction*

Number of genes	Modal frequency in binomial series	Total population (sum of binomial coefficients)	Standard deviation (S_{bi}) of $[1+(n+1)]^n$ (geometric) [1]	Population in modal class
	Number	*Number*		*Percent*
1		2	0.707	
2	2	4	.831	50.00
3		8	.933	
4	6	16	1.021	37.50
5		32	1.102	
6	20	64	1.171	31.25
7		128	1.237	
8	70	256	1.292	27.34
10	252	1,024	1.389	24.61
12	924	4,096	1.474	22.56
14	3,432	16,384	1.545	20.95
16	12,870	65,536	1.590	19.64
18	48,620	262,144	1.646	18.55
20	184,756	1,048,576	1.699	17.62
22	705,432	4,194,304	1.738	16.82
24	2,704,156	16,777,216	1.777	16.12
26	10,400,600	67,108,864	1.812	15.50
28	40,116,600	268,435,456	1.845	14.94
30	155,117,520	1,073,741,824	1.875	14.45
	Logarithm of number	*Logarithm of number*		
32	8.7789325	9.6329599		14.00
34	9.3680276	10.2350199		13.58
36	9.9578531	10.8370798		13.21
38	10.5483313	11.4391398		12.86
40	11.1393959	12.0411998	1.981	12.54
42	11.7309904	12.6432598		12.24
44	12.3230661	13.2453198		11.96
46	12.9155809	13.8473798		11.70
48	13.5084974	14.4494398		11.46
50	14.1017835	15.0514998	2.105	11.23
76	21.8383843	22.8782797		9.12
100	29.0038539	30.1029906	2.3104	7.96

[1] If this column is recalculated by arithmetic progression, the entire data will be useful in all cases of arithmetic or geometric interaction. Values not shown can be approximated by location on a curve connecting the given points, or calculated by the formula $S_{bi}^2 = \left(\frac{1+r^2}{2}\right)^n - \left(\frac{1+r}{2}\right)^{2n}$, where $r = (1+n)^{1n}$, n = genes.

TESTING GOODNESS OF FIT

The χ^2 test is the best gage of success in estimating the correct geometric mean. After three or four theoretical populations are calculated it is easy to see how an even better fitting population can be estimated by shifting the assumed geometric means lower or higher or by shifting the range of the binomial up or down. The theoretical population just calculated gives a χ^2 value of 71.678 with 8 degrees of freedom.[13] The shape of the frequency distribution then showed that an improvement could be made with a geometric mean at 4.4 kg. instead of 4.2. When this was done with the binomial $(1.2+16.1)^{14}$ the χ^2 value was 20.875 for 8 degrees of freedom. After again shifting the geometric mean to 4.5 kg. and the binomial to $(1.2+16.9)^{14}$ the χ^2 was reduced to 9.753, which for 8 degrees of freedom must be considered a very good fit with 5-percent tabular significance at 15.507. Success in finding better binomials is shown by the improvement made in regrouping raw data. For binomial $(1.2+14.7)^{14}$ the percentage modal class frequency is 22.7, but for $(1.2+16.9)^{14}$ it is 20.8, which is very close to the tabular value of 20.95 from table 6. Shifting the assumed mean to any other value than 4.5 kg. increased χ^2, and widen-

[13] Classes with frequencies below 5 should be combined with adjoining classes.

ing or contracting the binomial range beyond 1.2 to 16.9 increased χ^2; therefore the true geometric mean is bracketed with the mean and range thus found.

FINDING BEST ESTIMATE OF NUMBER OF SEGREGATING GENES

Data of table 4 show that the 490 weights of the F_2 population had a variance from total sources of 3.826 and the Northern Sweet parental line planted with it had an environmental variance of 1.446. Therefore, by the formula for a genetic standard deviation, S_g is 1.543. Table 6 indicates that the closest S_{bi} is 1.545 for the generalized binomial $(1+15)^{14}$, which has a geometric mean of 3.88 for comparison with the obtained population geometric mean of 4.5 kg.

It was learned that any assumed number of genes as well as 14 will give a bracketed χ^2 value, but only provided the geometric mean was 4.5 kg. The results of extending the assumed number of genes lower and higher than 14 is shown in table 5. The 15 trial binomials having the best fitting χ^2 value for even numbers 2 to 30 gave an average genetic standard deviation (S'_g) of 1.791. Value for S_{bi}, on the other hand, increased as the number of genes increased; consequently, the best estimate of gene number is that for which the difference between S'_g and S_{bi} is least; in the present case this is between 24 and 26.

The hypothesis of 25 genes may also be tested by calculating a proportion in which $\bar{x}$, or the adjusted genetic standard deviation, is to S_g of the obtained population as the obtained population geometric mean is to the generalized binomial geometric mean.

Calculation of $\bar{x}$ is as follows:

$$\bar{x}/1.543 = 4.5/3.88, \text{ in which } \bar{x} = 1.790$$

ESTIMATING NUMBER OF GENES SEGREGATING FOR WEIGHT IN BACKCROSS POPULATIONS

The two populations of F_1 Northern Sweet $\times$ Dove backcrossed to both parents were also analyzed for estimated gene number. Table 4 shows that the backcross to Northern Sweet had an environmental variance (S_e^2) of 1.518 estimated from the Northern Sweet planted with it and that 2.807 is the variance of the segregating population. The estimate of the genetic standard deviation (S_g) becomes $\sqrt{2.807 - 1.518}$, which equals 1.135. The indicated tabular binomial was $(1+7)^6$ whose geometric mean is 2.646 and whose S_{bi} adjusted for a frequency difference of 64 versus 490 is 1.166. The fitted binomial of the backcross population (fig. 5) is $(1.1+15.28)^n$, whose geometric mean is 4.1 kg. If the adjusted standard deviation of the generalized binomial (S_{bi}) is to be calculated by proportion, as for F_2, allowance must be made for the fact that only half as many genes are segregating in a backcross as in F_2, and the weight increment will be only half that obtained in F_2. Therefore, the value of $\bar{x}$ calculated by proportion will be twice the weight increase to be expected. The proportional adjusted value of $\bar{x}$ is calculated as follows: $\bar{x}$ is to 1.166 as 4.1 is to 2.646; $\bar{x}$ is 1.807. The increment is 0.641 (1.807−1.166), half of which is 0.321; therefore, 1.166 plus 0.321 is 1.487. This final value of $\bar{x}$ (1.487) corresponds to a tabular genetic standard deviation of 12 genes (table 6); consequently the fitted binomial is $(1.1+15.28)^{12}$ and the estimate of 12 genes agrees
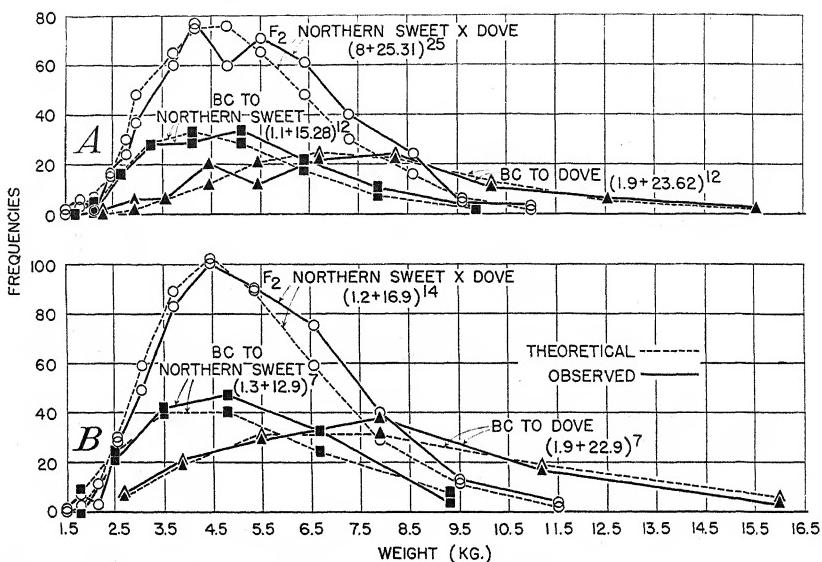
FIGURE 5.—Results of curve fitting between theoretical and observed populations of F_2 of Northern Sweet $\times$ Dove and of F_1 backcrossed to both parents, using two sets of bracketed binomials from table 5. *A*, The most probable binomials raised to the 25th and 12th powers. *B*, A random selection of other bracketed binomials shown in table 5 raised to the 14th and 7th powers

with the two estimates of 12 or 13 pairs of genes made from analysis of the F_2 data, where the best fitted binomials $(0.8+25.31)^{24}$ and $(0.7+28.93)^{26}$ bracketed the S_{bi} for 25 genes.

Analysis of the backcross to Dove is less satisfactory, however, as may be expected when the backcross parent is heterozygous. The gene number 12 was therefore arbitrarily adopted, and the population fitted to a curve based on the fitted binomial $(1.9+22.9)^{12}$, with population geometric mean of 6.6 kg. The results of curve fitting in all three of the populations of this cross are shown graphically in figure 5.

DISCUSSION

Variance analyses of the foregoing data confirm the observations of Weetman (*13*) and Rosa (*9*) regarding the relation of shape to sex and weight; and furthermore they indicate a strong probability of genetic linkage in both instances. Especially is this true in the case of the sex-shape interaction, where linkage values have been calculated for F_2 and backcross populations. The shape-weight interaction is not readily measured because of the presence of many genes for weight, probably 12 or 13 loci in the cross Northern Sweet $\times$ Dove, only 1 of which need be on the same chromosome with that for shape in order to account for the weak though highly significant coefficient calculated. The covariance data between shape and weight in this same F_2 population, -0.3128 for total sources of variance and -0.3755 for random error sources of variance, are in close agreement with the -0.34 and -0.41 found by Weetman in 2 different F_2 populations; but other segregating populations fail to show any significant degrees of correlation between shape and weight.

Such discrepant data for shape and weight are to be expected from cross to cross, because *Citrullus vulgaris* has 11 pairs of chromosomes, and the 12 or 13 pairs of genes indicated for the cross Northern Sweet × Dove are probably well scattered among the chromosomes. Furthermore, where multiple loci determine the effects of 1 character like weight the ones involved in 2 different crosses will hardly be the same.

The excessive χ^2 values for the locus Aa, shown in table 1 for most populations, require an adjustment to care for this distortion. Snedecor and Cox's adjustment of frequencies and sums for expected subclass numbers (*11*) was designed for such cases. In table 2 variances for weight and shape calculated from the original data are compared with adjusted values, and a general improvement from adjustment is evident. The only change in interpretation, however, concerns the variance in weight due to sex, where the original nonsignificant variance of 0.2056 became 14.0559, significant at the 1-percent point.

Calculations have shown that sex and weight are both probably linked with shape and should therefore be linked with each other. Consequently, the adjusted values are in harmony with expectations, and the mildness of the sex-weight interaction is explicable on the assumption that the sex and weight loci are on opposite sides of the shape locus and too far apart for the interaction to be strongly marked.

The positive skewness associated with F_2 populations interacting with geometric effects might at first be mistaken to indicate dominance of genes for small size. However, this occurs mostly because genetic intervals are smaller at the beginning of the range; hence the population tends to heap up at that part and spread out on the other end of the range. Class frequencies are the same in a binomial whether interaction is arithmetic or geometric, but the shape of the outline depends on the type of gene interaction; increase by a common number for arithmetic interaction gives uniform class intervals, and increase by a common rate for geometric interaction gives increasingly longer class intervals.

If measurements of the F_2 population under consideration were made in pounds rather than kilograms the geometric mean would have been about 9.2 pounds instead of 4.2 kg. and the S_g about 3.395 pounds instead of about 1.543 kg. The value 3.395 is so high that column 4 of table 6 cannot be entered even at 100 genes, and the most logical transformation would be from pounds to some unit of the metric system. Division of the mean and S_g expressed in pounds by the conversion factor 2.2 gives kilogram values more nearly comparable to the scale of tabular S_{bi} values.

The need already pointed out for adjusting theoretical and bracketed binomials to compensate for inevitable differences in spread was also stated by Powers (*8*) in his study of the sources contributing to environmental variances. Powers found that the experimental design should include both parents and the F_1 generation in each block. The environmental variance is calculated as the geometric mean of the variances of the three homozygous populations; in other words, each parental population occupies one of three different portions of the environmental range.

In the Northern Sweet × Dove populations used for this study, the Northern Sweet parent alone had sufficient seed for inclusion in all 10

blocks; consequently, the environmental variance (S_e^2) in this problem was assumed to be given by this one parent. For comparison, however, calculation of the S_e^2 from data of all 3 homozygous generations planted with the F_2 (see table 4) generation was made and found to indicate the segregation of 30 genes instead of 25 genes. This may mean that sampling the parental range at only 1 point instead of 3 points gave the best agreement with analysis by subtraction of S'_g average values in table 5 from S_{bt}. In any event, planting all 3 parental generations along with F_2 in each block would be very difficult if not impossible in most plant material.

Practically all the authors cited in this study agree that gene interaction in weight inheritance, at least, is geometric. Sinnott (*10*) states the argument as follows:

> Fruit size differences at maturity are due to several distinct and apparently independent processes. The production of new cells by division, the expansion of cells to their final size, the attainment of reproductive maturity (which affects the duration of cell division), and the development of the secondary cell wall (which affects the extent of cell enlargement) are four such processes. . . . If one gene tends to double the number of cells . . . and another to triple their volume, the combined effect of the two will be to multiply the volume of the fruit by six times.

Consequently, it is believed that a method of estimating total number of segregating genes in multiple-factor inheritance of a character like weight is significant on the grounds that if the main effects are geometric, the overlap between genetic classes smoothes out the effects of other phenomena like linkage. If the correct geometric mean is known, then the effective segregating range, together with the genetic standard deviation, may be calculated; and the most probable number of segregating genes is given by the adjusted value of the appropriate standard deviation of $[1 + (n+1)]^n$.

When 12 or 13 pairs of genes segregate in an F_2 generation, a backcross to a homozygous parent should segregate at the same 12 or 13 loci but only half as frequently as in F_2. Table 4, which gives weight constants for the generations planted with the F_2 generation, shows that the Dove parent was undoubtedly heterozygous for weight genes. Consequently, the tabular value of S_{bt} cannot show precisely whether the F_2 gene number involves 12 or 13 loci. Since the original F_1 was undoubtedly heterozygous, it is possible that another F_2 population from the same cross might show 11 or even 14 loci.

The population from the backcross to Northern Sweet is the only one from a backcross to a homozygous parent, and its number of segregating genes was estimated by adjusting the first trial generalized binomial by one-half the increment indicated by the method of adjusting the F_2 population of the same cross. This argument is supported by the fact that, when F_2 and backcross generations are adjusted, respectively, by the full and the half increment computed, the estimated number of segregating genes is 25 and 12 according to expectation.

SUMMARY

In the watermelon the 2 characters fruit shape (*O* elongate vs. *o* spherical) and plant sex habit (*A* monoecious vs. *a* andromonoecious) are shown to be linked with a cross-over value ranging from 0.136 ±0.029 for a backcross population of 140 plants to 0.207±0.010

for segregating F_3 populations totaling 2,085 plants and to 0.350 $\pm$0.035 for an F_2 population of 301 plants.

Fruit shape and fruit weight are significantly correlated in three groups of crosses (including backcrosses), in the best designed population of which $r = -0.313$ from total sources of variance and -0.863 for the four sex-shape Mendelian phenotypes; but the two characters fail to show significant correlation in some other crosses. Linkage estimates are impossible because of multiple-factor determination of weight.

Weight data distributed according to sex phenotypes show weakly significant or nonsignificant differences between the mean weights from the two sex types, but linkage between sex type and one of the weight genes is probable in the particular cross studied.

Number of genes segregating for weight inheritance in the cross Northern Sweet (about 3.2 kg.) $\times$ Dove (about 8.0 kg., heterozygous for weight) was estimated from the F_2 population at 25 genes and from the backcross to Northern Sweet at 12 genes.

Gene number estimation was made by comparison of the geometric mean and genetic standard deviation of the observed population with the geometric mean and standard deviation of the generalized binomial $[1 + (n+1)]^n$ when adjusted for differences in population frequency and spread.

When the true geometric mean of a segregating population is known, a good-fitting binomial can be made for any postulated number of genes; hence, the most probable number must be estimated according to the genetic standard deviation of the original uncorrected data.

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SOME FACTORS AFFECTING THE INSECTICIDAL ACTION OF PYRETHRUM EXTRACTS ON THE BEET LEAFHOPPER[1]

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INTRODUCTION

The beet leafhopper (*Eutettix tenellus* (Bak.)) is the vector of curly top, a disease of beets, beans, tomatoes, and other crops. Pyrethrum extract applied in oil as an atomized mist has been found to be very effective against the insect (*2, 4*),[3] and the practicability of this method for protecting sugar beets grown for seed under the mild winter conditions of the Southwest has been demonstrated (*7*). Owing to continued movements of the insect from desert breeding areas to the cultivated sections during the earlier and more susceptible stages of plant growth, it has been found extremely difficult to control curly top in beets grown for sugar (*4*).

The variability of the results obtained in field tests during the course of insecticide investigations on the beet leafhopper suggested that the insecticidal action of pyrethrum-oil sprays was being influenced by temperature or other weather factors. Several references in the literature on pyrethrum show that temperature may exercise an important effect on the insecticidal action of this material. Chevalier (*3*) found that the insecticidal effect of pyrethrum extract on cochylis caterpillars was much greater at 16° C. than at 36°, and Hartzell and Wilcoxon (*6*) reported that when rose chafer adults in a moribund state from pyrethrum intoxication were exposed to a higher temperature, the processes of recovery and death were both accelerated. According to Fleming (*5*), pyrethrum sprays were much more effective against the Japanese beetle in warm, sunny weather than under cool, cloudy conditions. Böttcher (*1*) tested pyrethrum extract both as a stomach poison and as a contact insecticide on the honeybee and found in each case that the toxic effect was greater at 20° C. than at 34.5°.

The present paper describes results of laboratory tests on the relative effectiveness of pyrethrum extracts under different controlled conditions of temperature and relative humidity. The chief purpose of the study was to ascertain the probable causes of variation in control of the beet leafhopper with pyrethrum-oil sprays.

[3] Italic numbers in parentheses refer to Literature Cited, p. 565.

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METHODS, APPARATUS, AND MATERIALS

The insects used in these experiments were adult females of the beet leafhopper collected from Russian-thistle (*Salsola pestifer* (A. Nels.)) near Twin Falls, Idaho. Some of the first collections were made with a sweep net, but this method was superseded by the use of the mobile collecting trap described by Douglass and Bean.[4] To avoid possible variation arising from differences in sex ratio of the groups of insects used in different experiments, female leafhoppers were used exclusively. The insects were collected 1 or 2 days before they were to be used in spray tests and were caged on a large beet plant in the laboratory during that time. The insects were selected at random in groups of 10, injured or obviously parasitized specimens being avoided, and were placed in smaller cages by means of a small suction tube. These cages of insects were then separated at random into the various groups to receive different treatments.

The cages (fig. 1, *C*) were 4 inches in length and about 2 inches in diameter and were made by cementing small sheets of cellulose acetate (gage 0.02 inch) in the form of cylinders and closing the ends with wire-mesh caps. A small brass grommet was placed in the center of the screen cap at one end of the cage to provide an opening. Small sugar beets grown in the greenhouse were used as host plants. Leaves used in the cages were trimmed to a common pattern to make them uniform in size and shape. The cages were mounted on the plants by thrusting a single leaf through the grommet and closing the opening with a split rubber stopper mounted around a bit of cotton on the petiole. The insects were introduced through a small hole punched in the sleeve portion of the cap and side wall of the cage, which was opened and closed by rotating the cap.

Standardized methods were used so that each group of insects would be sprayed in as nearly the same manner as possible. The methods were also designed to permit spraying under controlled temperature-humidity conditions and to eliminate the excess spray from the cabinet[5] to avoid fumigation effects. The equipment, which included an electric blower, a funnel, and pieces of flexible metal tubing, is illustrated in figure 1, *A*.

When the insects were sprayed under different conditions of temperature and humidity, the apparatus was placed in a controlled cabinet with the exhaust end of the spray duct protruding through a circular opening to the outside of the chamber (fig. 1, *A*). A small door in the cabinet permitted manipulation of the plant and insertion of cages in the device in preparation for spraying. The insects were sprayed in two ways, (1) while caged on the plants, and (2) before being placed on the plants. In the first method spraying was accomplished by bending the leaf petiole and inserting the cage through an aperture into a section of the tube that was opened and closed by rotating a metal sleeve (fig. 1, *D*). Opposing edges of the metal parts were padded with felt to permit closure of the opening without injury to the petiole. When the leafhoppers were to be sprayed before being placed on the plants, corks were placed in the grommet openings to confine the insects before and during spraying, and the cages were later mounted on the plant by the method just described.

[4] DOUGLASS, J. R., and BEAN, E. H. A MOBILE INSECT-COLLECTING TRAP. U. S. Bur. Ent. and Plant Quar. ET-146, 2 pp., illus. 1939. [Processed.]

[5] ANNAND, P. N., and HARRIES, F. H. TEMPERATURE-HUMIDITY CONTROLLED CABINETS FOR THE STUDY OF INSECTS. U. S. Bur. Ent. and Plant Quar. ET-159, 6 pp., illus. 1940. [Processed.]

For spraying the insects alone, the cage could be placed in the intake tube, or ahead of the suction fan, with the advantage that this portion of the system could be washed more thoroughly to remove spray residues (fig. 1, *B*).   After the cage had been inserted in either position the blower was started by a switch outside the cabinet, and
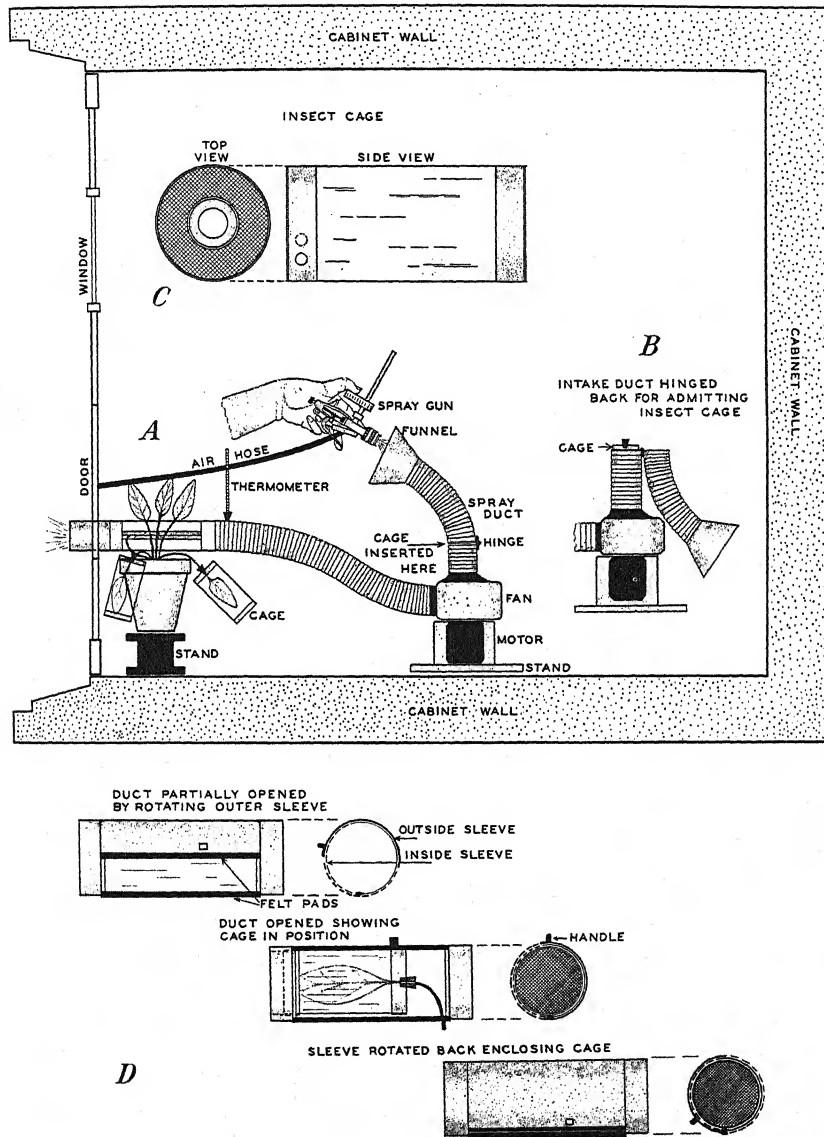

FIGURE 1.—Diagram illustrating methods and equipment used in spraying beet leafhoppers under controlled conditions of temperature and relative humidity: *A*, General arrangement of equipment as installed in a constant-temperature cabinet; *B*, intake duct opened for insertion of the cage; *C*, cage for holding 10 leafhoppers; *D*, method of inserting cage in spray duct near its outer end.

the spray material was directed into the funnel-shaped intake of the apparatus by means of a spray gun. In this way the spray mixed with conditioned air was conducted through the cage containing the insects and was passed to the outside of the chamber. The gun was charged with spray measured directly from a burette, was operated with compressed air at 40 pounds' pressure, and was carefully adjusted to atomize 1 ml. of the material in 10 seconds. To aid in maintaining uniformity, each spray application was checked with a stop watch. Except in the first experiment, when 0.5 ml. was used, the spray charge was 1 ml., or, in other words, 1 ml. was applied to each cage of insects. The spray gun was carefully adjusted for each of the different spray mixtures employed so that this quantity could be discharged in 10 seconds.

Dust charges were weighed on an analytical balance and were applied in the same way as the sprays by means of a special dust gun.[6] The duster was operated with compressed air and was adjusted to deliver a charge of 50 mg. in an interval of about 10 seconds.

The temperatures in the cabinets were controlled so as to deviate from the mean not more than half a degree on the Fahrenheit scale, and the relative humidity to deviate not more than about 1 percent. The blower was operated only during the time required to deliver the spray or dust charge so as to avoid affecting temperature and humidity in the cabinets by exhausting more conditioned air than was necessary to eliminate the excess material. The temperature of the spray entering the insect cages was checked with a thermometer (fig. 1, *A*) and also with a small thermocouple. Differences greater than 1 or 2 degrees from cabinet temperatures were not detected by either method.

One cage of 10 insects was included in each test, and the treatments of any given experiment were tested an equal number of times on any given day. The tests were repeated on different days until the desired number of replicates was obtained. Mortality counts were based on observations made 24 hours after the application of insecticides. This time interval was selected because preliminary tests had shown that at temperatures of 60° F., or above, it was sufficient to permit any recovery from sublethal dosages.

Each time the cages were used, they were thoroughly washed in warm soapy water to remove spray residues and then were rinsed and dried with clean towels.

Some of the experiments involved the transfer of the leafhoppers from one cage to another. This was accomplished by shaking them through a celluloid funnel fitted over open ends of the two cages. The leafhoppers were prevented from escaping while the funnel was substituted for the cap of the cage, and vice versa, by lightly shaking them downward.

The pyrethrum-oil sprays consisted of 1 part of a proprietary petroleum extract of pyrethrum to 500 parts of an oil base. The base of the sprays used in studies of the effects of temperature and humidity consisted of 2 parts of white kerosene to 1 part of oil B (table 1). In tests of the effect of different base oils the above mixture and kerosene alone were compared with similar mixtures in which oils C and D were substituted for oil B. Different proportions of white

[6] HARRIES, F. H. A DUST GUN FOR LABORATORY EXPERIMENTS WITH INSECTICIDES. U. S. Bur. Ent. and Plant Quar. ET-180, 2 pp., illus. 1941. [Processed.]

kerosene to oil B were also tested.   The pyrethrum-water spray contained 1 part of a commerical alcoholic extract to 400 parts of distilled water.   The dust was mixed from a commercial preparation of petroleum extract in diatomaceous earth in the proportion of 1 part of this material by weight to 9 of talc.

TABLE 1.—*Description of oils used in pyrethrum-oil sprays in experiments with the beet leafhopper*

Spray oil	Gravity, A. P. I.[1]	Viscosity, Saybolt Universal, at 100° F.	Flash point, Cleveland	Fire point, Cleveland	Minimum unsulfonated residue
		Seconds	*°F.*	*°F.*	*Percent*
A [2]		32			96
B		60–65			92
C	29. 0–33. 0	52–62	260	290	90
D	29. 5–32. 5	70–80	280	320	98

[1] American Petroleum Institute.
[2] White kerosene.

All materials were kept under favorable conditions to prevent deterioration during the study.   An analysis at the end of the period showed that there was 0.70 percent of pyrethrin I and 1.17 percent of pyrethrin II in the pyrethrum extract used in the oil sprays, 1.04 percent of pyrethrin I and 1.10 percent of pyrethrin II in the impregnated diatomaceous earth used in the dust mixture, and 0.62 percent of pyrethrin I in the water-spray extract.   The percentage of pyrethrin II in the water-spray extract could not be determined.

RESULTS AND DISCUSSION

EFFECTS OF EXPERIMENTAL TECHNIQUE

Preliminary experiments were conducted in which the leafhoppers were sprayed at five different temperatures with an oil mixture consisting of two-thirds white kerosene and one-third heavier oil (oil B) containing pyrethrum extract, and with the same oil to which no pyrethrum was added.   Insects treated with the pyrethrum-oil spray were included in two series that were sprayed (1) before being placed on the plants and (2) while caged on the beet leaves.   In these preliminary tests each cage of insects was treated with a spray charge of 0.5 ml., or only one-half that used in the later tests.

The results of these experiments, based on the means of 34 tests at 5 temperatures and the same number of comparable checks, are shown in table 2.   There was a tendency for mortality to be higher at the lower temperatures.   A slight decrease in mortality for a drop in the temperature from 60° to 50° F. tends to show a reversal of the trend, but the occurrence of a considerable number of apparently though not actually dead insects at 50° indicated that the observation period of 24 hours was not long enough to reflect the full action of the spray at this temperature.   Death or recovery from the effect of the spray was much more rapid at the higher temperatures, as was noted by Hartzell and Wilcoxon (6) in the case of rose chafer adults.

In the unsprayed lots mortality was very low, averaging 3.53 ± 0.55 percent.   Since these lots were in the cabinets at the time of the spraying operations, the low mortality indicates that there was no

important fumigating effect. There was a significantly higher mortality of 13.06 ± 1.21 percent in the lots sprayed with oil containing no pyrethrum, showing that the oil base alone had an insecticidal effect. The effect of the oil, however, was small as compared with the combined effect of the oil and the pyrethrum, as the latter resulted in an average mortality of 42.06 ± 2.08 percent when applied to the insects on the plants and 58.06 ± 2.16 when applied to the insects first. The relative effect of the base oil may be exaggerated in the present case since it was necessary to reduce the concentration of pyrethrum extract to less than one-tenth that used by Douglass, Wakeland, and Gillett (4) in field experiments.

TABLE 2.—*Mortality of beet leafhoppers held at indicated temperatures and 50 percent relative humidity both during and after the application of pyrethrum-in-oil spray and of oil spray without pyrethrum*

Temperature (° F.)	Mortality in—			
	Pyrethrum-oil spray [1]	Pyrethrum-oil spray [2]	Oil spray [2]	Unsprayed
	Percent	*Percent*	*Percent*	*Percent*
90	38. 24±4. 31	28. 53±3. 93	9. 41±1. 71	5. 88±1. 67
80	46. 76±4. 48	25. 29±3. 36	5. 59±1. 39	1. 76± .78
70	65. 59±4. 38	48. 24±4. 86	11. 76±2. 81	3. 24± .99
60	71. 76±3. 59	58. 53±3. 70	20. 88±3. 21	2. 06± .91
50	67. 94±4. 50	49. 71±4. 43	18. 24±3. 04	4. 71±1. 39
Difference required for significance ($P=$ 0.05)	12. 43	11. 92	7. 40	3. 48

[1] Sprayed in cages of leafhoppers before they were placed on plants.
[2] Sprayed in cages of leafhoppers after they were placed on plants.

A comparison of results for the two groups treated with the pyrethrum-oil spray shows a significant difference (16.00 ± 3.00 percent) that is evidently due to protection afforded by the beet leaf during spraying. This fact also indicates that a more nearly standard treatment was obtained when the leafhoppers were sprayed before the cages were placed on the plants, since the position of the leaf and the location of the insects would vary in different containers.

The effect on insect mortality of spray residues left on the beet leaves and in the cages was checked by the following procedure: 50 empty cages were mounted on plants and treated at room temperatures of 70° to 80° F. with a spray charge of 1 ml. The cages were then removed and placed on fresh or unsprayed leaves, and clean cages were placed over the sprayed leaves. Leafhoppers were counted into the cages in both series and also into a third series of clean cages placed on unsprayed leaves. The insects were then held in a controlled cabinet at 80° F. and 50 percent relative humidity for 24 hours before examination. The following results were obtained:

Treatment:	*Mortality (percent)*
Sprayed cages on unsprayed leaves	52. 60 ± 3. 71
Clean cages over sprayed leaves	4. 40 ± 1. 10
Clean cages over unsprayed leaves	3. 60 ± 1. 23

Mortality in the sprayed cages was significantly greater than in the other two groups, showing that spray residues in the cages have an important effect. A higher mortality was obtained on sprayed leaves than on unsprayed leaves, but the difference is not significant.

Since it was not convenient to treat all the unsprayed insects with the blower apparatus, possible effects of this difference in treatment of sprayed and unsprayed insects were checked by a comparison of insects exposed to the action of the blower as in spraying with an otherwise strictly comparable group that was not so treated. Both groups of 50 lots each were held at 80° F. and 50 percent relative humidity for 24 hours before examination. The following results show no significant effect of the difference in treatment:

Treatment:	Mortality (percent)
Unsprayed insects exposed in blower	2. 20 ± 0. 65
Unsprayed insects not exposed in blower	2. 00 ± . 57

EFFECTS OF TEMPERATURE

A summary of the results of 34 tests each with sprayed and unsprayed groups at 5 temperatures has been given in table 2. Statistical evaluation of the results by the method of analysis of variance showed that temperature had a highly significant effect on mortality in both series of tests of the pyrethrum-oil spray, as well as in the test of the base oil containing no pyrethum. A similar analysis of data on the unsprayed insects showed no significant effect of temperature on mortality.

A significantly lower mortality at the higher temperature was again obtained when a portion of the preceding experiment was repeated. These results, based on means of 50 tests at each temperature, are given in table 3, together with results of a similar experiment in which the spray was applied at the intermediate temperature of 80° F. in each test and the insects held for observation at the different temperatures.

TABLE 3.—*The influence of temperature at 50 percent relative humidity on the mortality of beet leafhoppers treated with pyrethrum-in-oil spray while caged on plants*

TREATED AT DIFFERENT TEMPERATURES AND HELD FOR OBSERVATION AT THE SAME TEMPERATURES

Temperature (° F.)	Mortality in 24 hours, of—	
	Sprayed insects	Unsprayed insects
	Percent	*Percent*
100	20. 00±2. 23	1. 80±0. 67
90	23. 80±2. 46	4. 00± . 89
80	42. 00±3. 52	1. 40± . 49
70	53. 00±3. 55	1. 80± . 54
60	46. 80±3. 93	3. 20±1. 15
Difference required for significance (*P*=0.05)	9. 20	2. 26

TREATED AT 80° F. AND HELD FOR OBSERVATION PERIOD AT DIFFERENT TEMPERATURES

100	32. 80±2. 76	2. 00±0. 69
90	27. 40±3. 52	1. 40± . 49
80	40. 80±3. 10	1. 40± . 63
70	54. 80±3. 30	1. 40± . 57
60	53. 00±3. 31	. 80± . 48
Difference required for significance (*P*=0.05)	9. 29	1. 66

The similarity of the two sets of data in table 3 indicated that the lower mortality observed at higher temperatures was more closely associated with the temperature during the period of observation than with that prevailing at the time of spray application. Accordingly, it seemed that this lower mortality might be due largely to the effects of temperature upon the physiology of the sprayed insects rather than to its effect on the physical character and mode of action of the spray.

The effect of temperatures prevailing at the actual time of spray application was tested more directly by spraying leafhoppers at different temperatures and humidities and immediately transferring them all to clean cages held at 80° F. and 50 percent relative humidity. The transfer was accomplished in about 30 seconds. The results of 50 tests at each temperature as summarized in table 4 show that a significantly higher mortality of leafhoppers was obtained at 100° than at 60° F. This is an effect directly opposite to that produced when the insects were held at different temperatures after spraying.

In a later experiment the insects were sprayed at room temperature (70° to 80° F.) before they were placed on plants in clean cages and held for observation at different temperatures and humidities. The insects were transferred to clean cages within 10 seconds after being sprayed. This precaution was taken in order to eliminate the possible influence of spray residues. The effect of spray residues was also studied under the different conditions by placing unsprayed insects in sprayed cages. The results of 50 tests under each condition are summarized in table 5.

TABLE 4.—*Mortality of beet leafhoppers sprayed with pyrethrum-in-oil at different temperatures and relative humidities and held for observation at 80° F. and 50 percent relative humidity*

Conditions during spraying		Mortality in 24 hours
Temperature (° F.)	Relative humidity	
	Percent	*Percent*
100	80	69.80±2.84
100	20	70.60±2.63
60	80	53.40±2.85
60	20	53.00±2.81
Difference required for significance (P=0.05)		7.98

TABLE 5.—*Mortality of beet leafhoppers sprayed with pyrethrum-in-oil at room temperatures and held in clean cages under several conditions of temperature and humidity, and mortality when unsprayed but held in sprayed cages*

Conditions after spraying		Mortality of—		
Temperature (° F.)	Relative humidity	Sprayed insects in clean cages	Unsprayed insects in sprayed cages	Unsprayed insects in clean cages
	Percent	*Percent*	*Percent*	*Percent*
100	80	28.80±3.21	15.60±1.94	5.80±1.20
100	20	35.60±3.13	12.00±2.22	11.00±1.88
60	80	65.60±2.40	18.40±2.58	.80± .48
60	20	81.40±2.00	28.60±3.46	1.60± .71
Difference required for significance (P=0.05)		7.83	7.51	3.43

The data show that a highly significant increase in mortality of the treated insects also occurred at the lower temperature when the factor of spray residues in the cages was eliminated. Mortality of unsprayed insects placed in sprayed cages was significantly greater in most cases than in the corresponding checks, and the analysis of variance shows a highly significant influence of temperature on the toxic effect of the residues.

In view of the foregoing results, the efficiency of pyrethrum sprays in the field appears to depend to a considerable extent upon the time of application with respect to the diurnal temperature gradient. It is concluded that, other conditions being favorable, the best time for spraying is during the late afternoon or evening when temperatures are relatively high and are followed by lower temperatures during the night. Temperatures during and following morning applications are not so favorable, as they are relatively low in the early morning and rise during the day. The rate of decline in temperature after application should also be important.

EFFECTS OF HUMIDITY

The experiment summarized in table 4 demonstrated that there was no appreciable difference in the mortality of leafhoppers sprayed at 80 percent relative humidity and that of those sprayed at 20 percent. This indicates that the effectiveness of pyrethrum spray is not materially influenced by the relative humidity existing at the time of application.

Studies of the effect of temperature and humidity following application of the spray showed an appreciably greater kill at the lower humidity at both temperature extremes (table 5), and the analysis of variance shows a highly significant effect of moisture with no evidence of an interaction or significant difference in the relative influence of humidity under different temperatures. In these tests the possible influence of spray residues was excluded by transferring the insects to clean cages before they were placed under the different controlled conditions.

Further studies of the effect of the pyrethrum-oil spray under different conditions of relative humidity were conducted by treating the leafhoppers at a series of different humidities at the same temperature in one experiment and at high and low humidities at two temperatures in another experiment. A total of 50 tests was made at each humidity in the first experiment and 100 tests at each condition of temperature and humidity in the second. Insects in both experiments were sprayed while caged on the plants at different humidities and were held under these same humidities during the 24-hour observation period.

An analysis of variance of the results given in table 6 shows a highly significant influence of relative humidity, there being a progressive increase in mortality with successively lower humidities. This effect of moisture appears relatively less pronounced than that of temperature shown in previous experiments, since a difference of only 12.40 percent mortality occurred under the extreme conditions of 10 and 90 percent relative humidity. The influence of humidity was more in evidence at 60° F. than at 100°. The results also supported those of previous experiments by showing a higher mortality at 60° than at 100°.

TABLE 6.—*Mortality of beet leafhoppers caged on plants at different relative humidities and temperatures during and after the application of pyrethrum in oil*

| Temperature (° F.) | Relative humidity | Mortality of— | |
		Sprayed insects	Unsprayed insects
	Percent	*Percent*	*Percent*
	10	88. 80±1. 66	1. 20±0. 69
	30	86. 40±1. 78	1. 20± . 46
100	50	86. 20±1. 87	1. 00± . 42
	70	81. 40±2. 23	2. 60± . 89
	90	76. 40±2. 77	1. 20± . 54
Difference required for significance (*P*=0.05)		6. 03	1. 78
100	20	87. 40±1. 49	6. 20±0. 86
100	80	86. 90±1. 16	6. 30± . 95
60	20	96. 90± . 56	6. 10±1. 46
60	80	93. 00±1. 02	5. 00± . 88
Difference required for significance (*P*=0.05)		3. 12	3. 00

EFFECTS OF THE BASE OIL ON TOXICITY OF PYRETHRUM-OIL SPRAYS AT HIGH TEMPERATURES

The effect of differences in the ratio of kerosene to the heavier oil (oil B) in spray mixtures was studied by spraying the leafhoppers, in the absence of plants, at room temperatures of 70° to 80° F. and transferring them immediately to clean cages on plants in a cabinet maintained at 100° F. and 50 percent relative humidity. Sixty-four replicates were made of each treatment. As shown in table 7, there was a slight but progressive increase in mortality with the mixtures

TABLE 7.—*Mortality of sprayed beet leafhoppers as influenced by the proportion of kerosene in the spray mixture*

| Ratio of kerosene to oil B | Mortality of insects sprayed with oil mixture— | |
	Containing pyrethrum	Not containing pyrethrum
	Percent	*Percent*
1:3	50. 47±2. 64	24. 53±2. 39
1:1	54. 84±2. 84	29. 69±2. 78
3:1	59. 84±2. 58	19. 69±2. 01
Difference required for significance (*P*=0.05)	7. 68	6. 88

containing pyrethrum as the ratio of kerosene to heavier oil increased. Data on the relative effect of the same oil mixtures containing no pyrethum fail to show this trend.

The following results, based on 64 replicates, were obtained when pyrethrum extract was used in a base of white kerosene and with different summer spray oils in the proportion of 2 parts of kerosene to 1 part of the heavier oil:

Type of oil mixed with white kerosene:	Mortality (percent)
None (kerosene alone)	59. 22±2. 95
B	47. 81±2. 67
C	37. 97±2. 64
D	40. 47±2. 43
Difference required for significance (*P*=0.05)	7. 63

The conditions of the experiment were the same as those described for the preceding experiment. There was a mortality of 8.13 ± 1.51 among comparable unsprayed insects. These data show greater differences than those included in table 7 and suggest the same trend since the spray with the kerosene base was most effective and that containing the heaviest oil was the least effective. Since in these tests at 100° F. the pyrethrum extract was more effective in the lighter-base oils, the efficiency of pyrethrum-oil sprays evidently cannot be increased at higher temperatures by using heavier or more viscous oils.

EFFECTS OF TEMPERATURE AND HUMIDITY ON THE INSECTICIDAL ACTION OF PYRETHRUM EXTRACT APPLIED IN A WATER SPRAY

Because of the important influence of temperature on the toxic action of pyrethrins shown in studies of pyrethrum-oil sprays, tests were conducted to determine whether the effects of temperature would be similar with pyrethrum-water sprays. The leafhoppers, in the absence of plants, were sprayed at room temperatures of 70° to 80° F. and immediately transferred to different conditions of temperature and humidity. As a check, comparable treatments were made with distilled water instead of the pyrethrum-water mixture.

Results obtained in 50 replicates of this experiment are summarized in table 8. As was found in similar tests with pyrethrum-oil sprays, these data show a much greater mortality at the lower temperature than at the higher one. Differences in atmospheric moisture had no significant effect. There was no appreciable mortality in the water-sprayed checks. Possible contributing effects of spray residues in the cages were checked by additional tests in which some of the insects were transferred to clean cages immediately after the spraying. The mortality was 19.80 ± 2.20 percent among the insects transferred to clean cages and 30.80 ± 2.81 percent among those not transferred. Among comparable unsprayed insects the mortality was 5.60 ± 1.55 and 5.60 ± 1.10.

TABLE 8.—*Mortality of beet leafhoppers sprayed with pyrethrum extract in water under room conditions and held at different temperatures and humidities during the observation period*

Conditions after treatment		Mortality in—	
Temperature (°F.)	Relative humidity	Pyrethrum-water spray	Water spray (check)
	Percent	*Percent*	*Percent*
100	80	24.20±2.52	5.40±1.33
100	20	20.00±2.32	4.40±.99
60	80	77.80±1.88	1.00±.51
60	20	82.40±1.82	1.60±.65
Difference required for significance (P=0.05)		6.18	2.66

EFFECTS OF TEMPERATURE AND HUMIDITY ON TOXICITY OF PYRETHRUM EXTRACT APPLIED AS A DUST

Some additional tests were conducted to determine whether temperature would have an effect on toxicity of pyrethrum extracts applied in dust form similar to that of pyrethrum extracts applied as a spray. The leafhoppers were treated with a charge of 50 mg. of

dust at room temperatures (70° to 80° F.) and were transferred immediately to clean cages and placed on the plants under different conditions of temperature and humidity.   Comparable check lots of insects were dusted with talc alone.

Results of the tests given in table 9 show a marked increase in insecticidal effect at lower temperatures, similar to that in the case of the sprays.   Analysis of the data shows a highly significant effect of temperature but no significant effect of relative humidity or of an interaction of temperature and moisture factors.   Data on the checks dusted with talc show no significant effect of temperature or humidity.

TABLE 9.—*Effects of pyrethrum extract on beet leafhoppers when it was applied in a dust under room conditions and the insects were held at different temperatures and humidities during an observation period of 24 hours*

Conditions after treatment		Mortality after application of—	
Temperature (°F.)	Relative humidity	Pyrethrum-talc dust	Talc dust
	Percent	*Percent*	*Percent*
100	80	32. 60±2. 71	4. 20±1. 10
100	20	37. 80±3. 09	4. 60±1. 14
60	80	92. 00±1. 17	8. 00±1. 44
60	20	88. 40±1. 75	5. 20±1. 14
Difference required for significance (*P*=0.05)		6. 62	3. 48

SUMMARY

Effects of temperature and humidity on the insecticidal action of pyrethrum extracts against the beet leafhopper (*Eutettix tenellus* (Bak.)) were studied in laboratory tests under controlled conditions. The insecticides were applied by placing the leafhoppers in screen-capped, cylindrical cages enclosed in a tubular air duct through which uniform charges of spray or dust materials were passed by means of an electric blower.

The effect of temperature was studied under the following conditions: When the leafhoppers were (1) sprayed with pyrethrum extract in oil at different temperatures and then held for 24 hours at these same temperatures, (2) sprayed at a common temperature and then held at different temperatures, (3) sprayed at different temperatures and all held at 80° F., and (4) sprayed in one set of cages and immediately transferred to clean cages to eliminate effects of spray residues left on the cage.   These tests showed that the mortality of leafhoppers sprayed with pyrethrum in oil could be increased by raising the temperature at the time of application but could be increased to a greater extent by lowering it after application.

As a practical application of these findings, it may be inferred that the best results in control would be obtained by treatments made in the late afternoon, which would naturally be followed by lower temperatures at night.

Mortality was higher among the insects held at the lower percentages of relative humidity for the observation period, but the effect of humidity was much less pronounced than that of temperature.

In a series of tests on the effect of the oil base of the pyrethrum-oil spray it was found that greater efficiency was obtained with the lighter oils, that with kerosene alone being the highest.

Tests of a pyrethrum-water spray showed a greater efficiency at the lower holding temperature, with no significant effect of humidity.

The effect of pyrethrum when applied as a dust was similar to that when applied as a spray, in that it was most effective at the lower temperatures. Variations in humidity caused no significant changes in the efficiency of the dust.

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